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CORRIGENDA

Page 40, Paragraph 1, 4th line :
    for Fig. 2   *read* Fig. 10

Page 40, Paragraph 2, 1st line :
    for Fig. 1   *read* Fig. 9

Page 40, Paragraph 3, 9th line :
    for Fig. 1   *read* Fig. 9

Plate I, facing page 42 :
    for Fig. 1   *read* Fig. 9
    for Fig. 2   *read* Fig. 10

    Page 45, Table I, columns 4 and 5 :

for			*read*		
$3 \cdot 654$	$0 \cdot 346$		$3 \cdot 654$	$0 \cdot 346$
$\pm 0 \cdot 019$	$\pm 0 \cdot 027$		$\pm 0 \cdot 027$	$\pm 0 \cdot 019$
$3 \cdot 877$	$0 \cdot 877$		$3 \cdot 877$	$0 \cdot 877$
$\pm 0 \cdot 023$	$\pm 0 \cdot 032$		$\pm 0 \cdot 082$	$\pm 0 \cdot 058$
$3 \cdot 960$	$0 \cdot 154$		$3 \cdot 960$	$0 \cdot 487$
$\pm 0 \cdot 012$	$\pm 0 \cdot 010$		$\pm 0 \cdot 036$	$\pm 0 \cdot 026$

Page 47, Fig. 1, about the middle :
    for $k = \pm 17$ *read* $k = \pm 1 \cdot 1$
    for $k = \pm 12$ *read* $k = \pm 1 \cdot 2$

Page 49, Table II, columns 3 and 4, against (*a*) Flowering
    for $\pm 0 \cdot 110$ *read* $\pm 0 \cdot 160$
    for $\pm 0 \cdot 160$ *read* $\pm 0 \cdot 110$

Plate II, facing page 64 .
    for Fig. 1   *read* Fig. 2
    for $\times 1$ *read* $\times \frac{1}{2}$
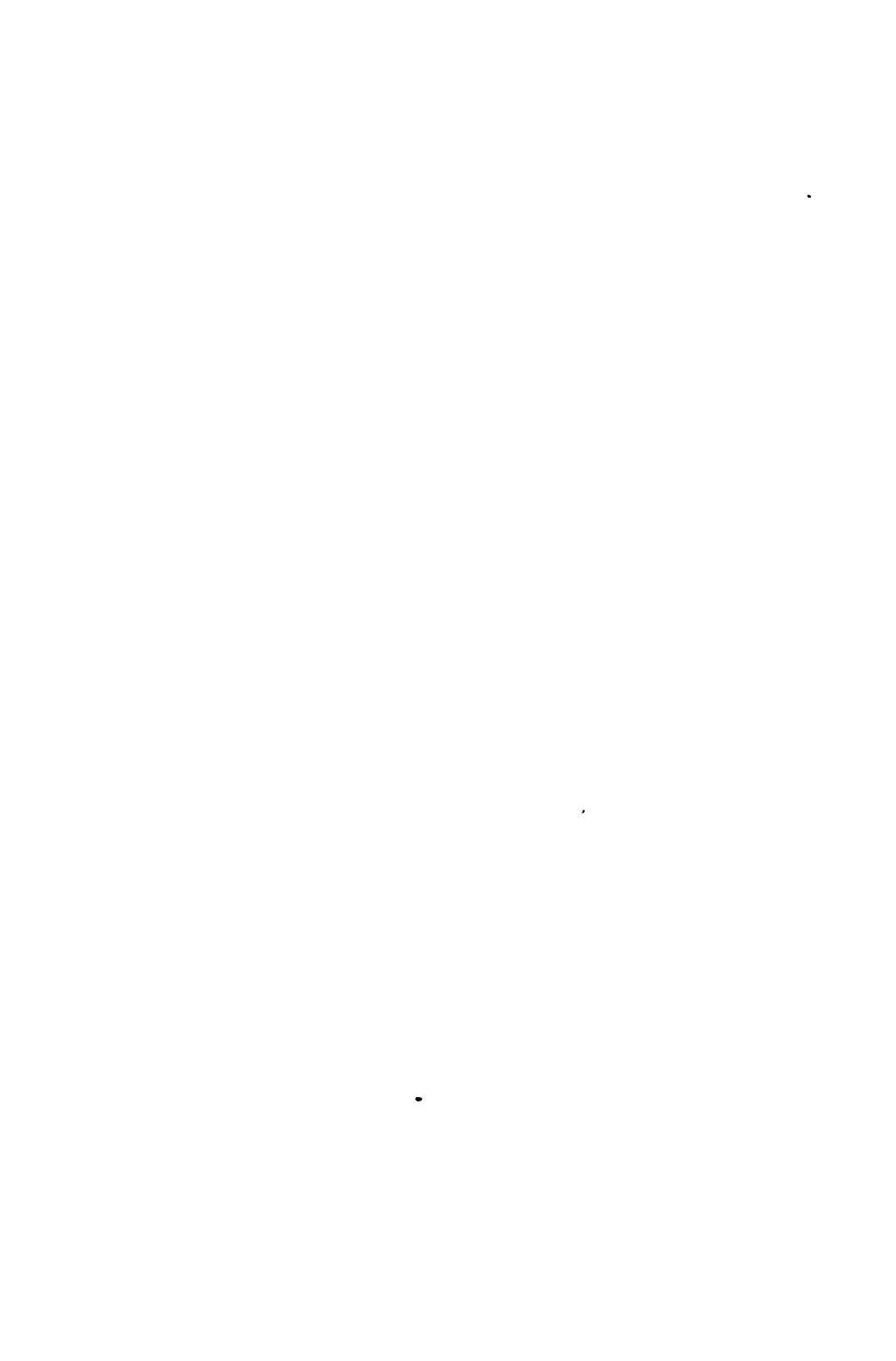
THE BIOLOGICAL SPECTRUM OF THE ALLAHABAD FLORA

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1. INTRODUCTION

IN 1905 Raunkiaer published the first comprehensive account of a Life-form System of plants which remains to-day in principle, the same. This system is simple, based mainly on *only one single feature* namely " the protection of the bud of the shoot-apices to the unfavourable season ". His main task was to describe a region in terms of the plant world. He believed that the plant climate can be characterised by a statistical survey of the life-forms.[14]

Borgesen[3] studied the vegetation of Dwarka with reference to Raunkiaer's life forms and statistical methods, and recently Bharucha and Ferreira[12] gave an account of the Biological Spectra of Madras, Matheran, and Mahabaleshwar.

Except the work of Dudgeon[7] and the author's, *Flora of Allahabad,*[12] no work on the vegetation of this place has been done so far. Therefore, it was thought desirable to make a bio-statistical study of the flora on the basis of Raunkiaer's life-form system. This study is restricted to an area of about ten miles in radius with Allahabad as the centre. The present work on the life-forms is based not only upon the above flora, which is fairly exhaustive but help was also taken from nos. 1, 5, 6, 8, 9, 10 and 11 of the bibliography. ·

2. PHYSICAL FEATURES

Topography.—Allahabad is situated at 25° 26' N. latitude and 81° 52' E. longitude at the junction of the Ganges and the Jumna rivers. It is 319 ft. above sea-level. The area dealt with forms a part of the floristic subdivision of India, known as the Upper

Gangetic Plain. The soil is all alluvial, deposited within the recent geological times. It ranges from sand through a mixture of sand and clay, to a fine clay. The older alluvium contains deposits of $CaCO_3$ in irregular nodules called *Kankar*. Both the rivers have been depressed in the recent past, so that during the highest floods, the surrounding plains are not covered with water. Except where dissected by deep ravines, the surrounding places are almost level. Here and there are slight natural depressions which become shallow lakes, some of them drying up during summer season, others remain covered with water. On the whole the soil presents a very uniform substratum for the growth of the vegetation.

Climate.—Allahabad, as it is situated, has a strongly periodic climate characteristic of the tropical region.

Rainfall.—Both the Bay of Bengal and Arabian Sea branches of the S.W. monsoon current contribute to the rainfall of the area. The mean annual rainfall is 39·06 inches. The records of the monthly and annual rainfall are given below in Table I and the corresponding graph in Fig. 1.

From Table I it will be seen that about 94% of the rainfall occurs from June to October while only about 3% from January to February. During the monsoon the rains are at times torrential. After usually heavy rains, the level areas become vast shallow seas.

Temperature.—Table II shows the maximum, minimum and mean monthly temperatures for the year. The temperature exhibits a large range between winter and summer and between day and night, despite the fact that it is barely outside the tropics. The lowest temperature is in December 47·7° F. and then there is a rapid rise to a maximum of 106·6° F. in May.

On rare occasions in winter the temperature may go down below freezing point but these exceptional low temperatures are of little importance in determining either the character or the composition of the vegetation. The highest recorded temperature was 119·8 on June 19, 1878.

Humidity.—The mean monthly and annual humidity is given in Table III.

The lowest humidity is in the month of April and May when the temperature is maximum, and reaches its maximum 85% in August. From June onwards the percentage increases.

Wind.—During most of the year the winds blow in fits. During April, May and June, beginning from about 10 A.M. and continuing till 6 or 7 P.M., there is a strong hot wind from N.N.W. locally called the '*lu*'. Often it continues throughout the night for a couple of days in the season.

Climatic seasons.—From the foregoing it is clear that the climate is markedly periodic and can be divided into 3 seasons: (1) Rainy season, (2) Winter season and (3) Summer season.

TABLE I— *Rainfall*

Mean Rainfall	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
In inches	0·76	0·58	0·31	0·15	0·34	4·96	11·71	11·70	5·67	2·32	0·33	0·23	39·06
In cm.	1·94	1·48	0·79	0·32	0·87	12·65	29·86	29·84	14·46	5·92	0·84	0·59	99·1

TABLE II— *Temperature*

Temperature	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Maximum in °F.	74·4	79·5	91·9	102·8	106·6	102·1	92·8	90·0	91·5	91·1	83·4	75·7
Minimum in °F.	48·0	51·9	61·7	72·0	79·6	82·7	79·8	78·6	76·9	67·5	55·3	47·7
Mean in °F.	61·2	65·7	76·8	87·4	93·1	92·4	86·3	84·3	83·95	78·8	69·35	61·7
Mean in °C.	16·2	18·7	24·9	30·7	33·9	33·5	30·1	29·0	28·8	26·0	20·9	16·5

TABLE III— *Humidity*

Humidity	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual
Mean %	68	67	47	36	42	61	80	85	80	67	68	75	65·5

Corresponding with the climatic seasons, there are three distinct vegetational seasons.

Rainy season begins from about the middle of June when the first showers of rainfall and lasts till the end of September. It is characterised by high rainfall, low insolation, high temperature and high humidity. The rainy season merges gradually into the winter season which extends from begining of October to the end of February. It is characterised by low rainfall, high insolation, low temperature and relatively high humidity. This merges into the summer season extending from beginning of March to the middle of June. It is characterised by high insolation, high temperature, low humidity and strong winds.

3. THE HYDROTHERM FIGURE

The hydrotherm figure for any region, according to Raunkiaer, is a figure showing the relationship between the temperature curve, plotted in degrees Centigrade and the precipitation (rainfall) curve, plotted in centimetres in the same graph. By combining the above data of the normals of rainfall and temperature of Allahabad, the hydrotherm figure is obtained (Fig. 1). In Fig. 2, the normals of rainfall and relative humidity are plotted in the same graph.

From Tables I, II and III and Figs. 1 and 2, it is clear that the mean temperature varies between $16 \cdot 2°$ C. in January and $33 \cdot 9°$ C in May and June. The temperature curve shows a conspicuous trough in the months of December and January when the

Months of the year
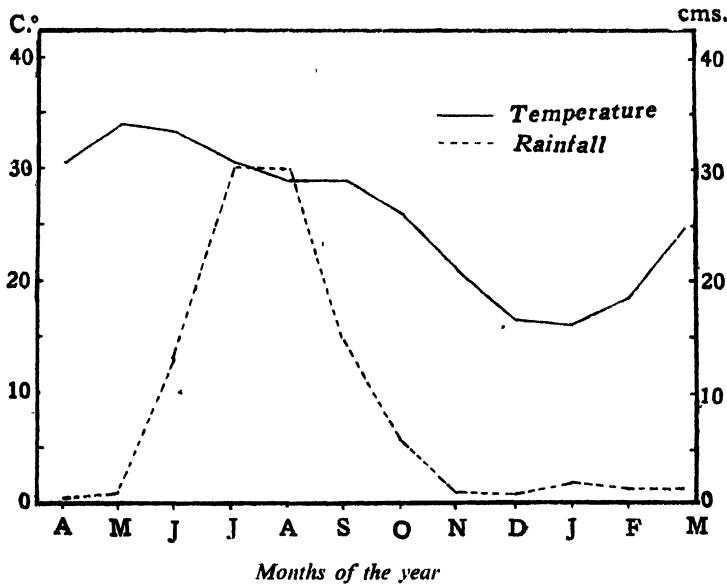
Fig. 1. Hydrotherm figure
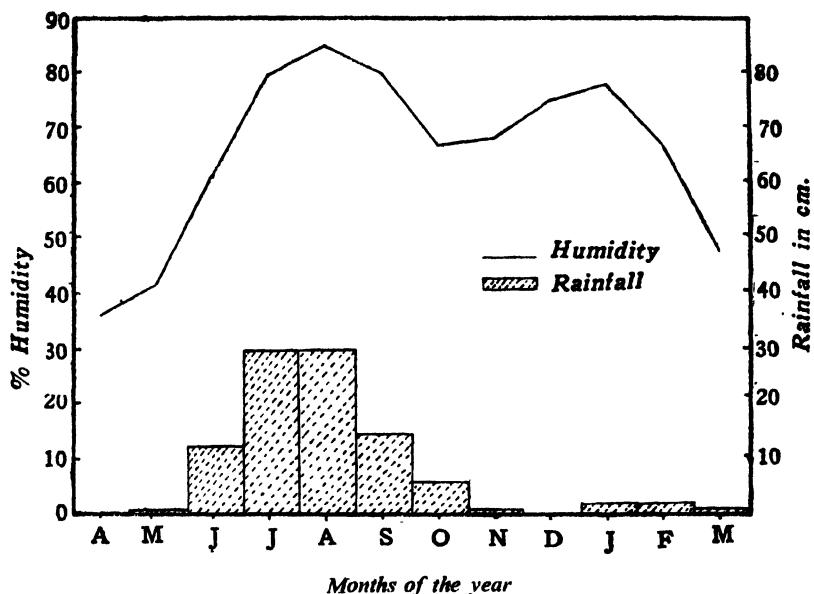
Fig. 2. Humidity and rainfall curves

temperature goes down to $16 \cdot 2°$ C. The rainfall ranges from a little above 0 to about $29 \cdot 9$ cm. in July when it reaches its maximum. The precipitation curve also shows two troughs, one in November and December and the other in March–May, when the rainfall is a little above zero. As regards humidity, it is the lowest in April and the maximum in August.

The trough of the precipitation curve occurs at a different season from that of the temperature curve, so we get a dry summer and a more or less humid winter.

Thus it will be seen that the climatic factors favour the presence of two growth seasons, one during the rainy season and the other during the humid winter season, i.e., January and February. The unfavourable season is from March to the middle of June when the mean rainfall is only a little above zero.

4. THE BIOLOGICAL SPECTRUM

Such sub-tropical regions, with a dry summer, slightly humid winter and a pretty long rainy season in between the two seasons, are bound to be characterised by the preponderance of the annuals. In the terminology of Raunkiaer, they would be characterised as having Therophytic plant-climate. According to Raunkiaer,[13,14] Therophytes which occur in sub-tropical region survive the unfavourable season in the form of seed and complete their life-cycle within a single favourable season. That this is so is borne

out by the biological spectrum given in Table IV. Here out of the ten life-forms of terrestrial plants of Raunkiaer, I have made use of only four main types and broken up the Phanerophytes into Megaphanerophytes, Microphanerophytes, Nanophanerophytes and Lianas and grouped the rest of them, such as the succulent-stemmed, epiphytic and parasitic Phanerophytes, into one sub-group.

TABLE IV*

Regions	Number of species	The percentage distribution of the species among the life-forms								
		L	E and S.	MM.	M.	N.	Ch.	H.	Cr.	Th.
Normal	1,000	..	5	8	18	15	9	26	6	*13*
Allahabad	628	3·1	2·7	3	17·6	11·6	9·2	3·4	7·8	*41·6*
Death Valley, California	294	..	3	..	2	21	7	18	7	42

* L=Lianas, E. and S.=Epiphytes and Succulents, MM.=Megaphanero-phytes, M= Microphanerophytes, N=Manophanerophytes, Ch= Chamæphytes, H=Hemicryptophytes, Cr=Cryptophytes, *i.e.* Geophytes, Helophytes, Hydrophytes, and Th=Therophytes.

In the above table are given the Biological Spectra of Allahabad, Death Valley, California, together with the Normal Spectrum. It will be seen that the life-form which exceeds most in the percentage number of plants in the spectrum for Allahabad is Therophyte 41·6% which is more than three times that of the Normal Spectrum (13%).

The groups next in importance respectively are the Micro-phanerophytes, the Nanophanerophytes, the Cryptophytes, and the Chamæphytes, which form 17·6%, 11·6%, 9·2% and 7·8% respectively of the total number of plants. Out of these the percentages of only the Cryptophytes and Chamæphytes exceed the corresponding figures in the Normal Spectrum, as can be seen from Table IV. Therefore the important life-forms for consider-ation are the Therophytes, Cryptophytes and Chamæphytes.

By applying Raunkiaer's formula, the following figures for the three life-forms are obtained :—

Thero.   Crypto.   Chamæ.
3·2   :   1·3   :   1·02

Thus the plant-climate may be characterised as Therophytic.

The percentage number of the Therophytes in Allahabad is almost the same as in the spectrum of Libyan Desert or Death Valley, California, which according to Raunkiaer, have a thero-phyte plant-climate. Dudgeon[7] also concluded in his study of the ecology of Allahabad that the annuals dominate the other plant

forms especially during the rainy and winter seasons and they are also present during the summer season, though the number is small.

From the above it is evident that according to Raunkiaer's terminology, Allahabad has Therophyte-plant-climate.

5. SUMMARY AND CONCLUSIONS

1. The bio-statistical study of the flora of Allahabad was made on Raunkiaer's life-form system.

2. The climate is markedly periodic and the whole year is divisible into three distinct seasons—the rainy, winter and summer seasons.

3. The biological spectrum was studied in correlation with topographical and climatic features. ·

4. The area shows decidedly a therophytic plant-climate with about 42% therophytes. This is confirmed by the results obtained by Dudgeon on the ecological study of this area.

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VARIATION IN THE RATE OF RESPIRATION OF A GERMINATING SEED

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EXTENSIVE work on respiration has been done by several investigators, and many types of respirometers have been designed and recommended for estimating this activity in plants. The kinds of micro-respirometers constructed [Osterhout and Haas (1917) and Lund (1919)] and the methods recommended appear to be too complicated. The indicator method of Osterhout (1918) although efficient needs very careful handling. Davis (1925) has designed an apparatus which works on the manometer principle but the estimation of carbon dioxide is done only after 24–48 hours of respiration. The several germinating seeds likely to be introduced at a time, the possibility of accumulation of carbon dioxide in the vicinity of seeds and the long period after which the estimation is made happen to be the drawbacks in this case. A more recent contribution to this subject is by Brown (1942) who has designed an apparatus and studied the rate of gaseous exchange in the seed and cotyledons of *Cucurbita pepo*. The smallest quantity recorded by him happens to be 1/100 c.c. The duration of each one of his experiments was 48 hours during which period four estimations are made, these being at intervals of 18, 24, 42 and 48 hours from the time of starting the experiment. According to the author each estimation takes about 10 minutes, or more for greater accuracy, and necessitates certain corrections in the volume for the time lost during estimation. In all these investigations the readings are taken at long intervals and no effort seems to have been made as yet to record this activity at very short intervals. It was this which made the author to study this activity in the germinating seeds. For this work a special kind of respirometer had to be designed on the 'float and manometer' principle (Krishna Iyengar, 1942 b), and this in combination with the optical lever constructed by the author makes it possible to record very small volume of gases. The simple construction, high magnification, easy handling, efficient working and lastly direct observation were the points in view during the construction of the apparatus (Fig. 1). Strong caustic soda solution is used for the ready absorption of carbon dioxide evolved. Miller (1931) is of opinion that ' the use of a strong solution of an alkali in the apparatus has a disadvantage, since the ready absorption of carbon dioxide by the alkali introduces large changes in pressure within the closed apparatus thereby affecting respiration '. In the present case the bulk of respiring

2

material is very small, the period of investigation is short, the difference in pressure is insignificant and even then provision is made to bring up the pressure to normal. The following is an account of the apparatus and the observed variation in the rate of respiration.

APPARATUS AND ITS WORKING

The trough of water (A) is meant to maintain a constant temperature in the specimen tube by directly absorbing any heat evolved during respiration. A film of oil is introduced to remove
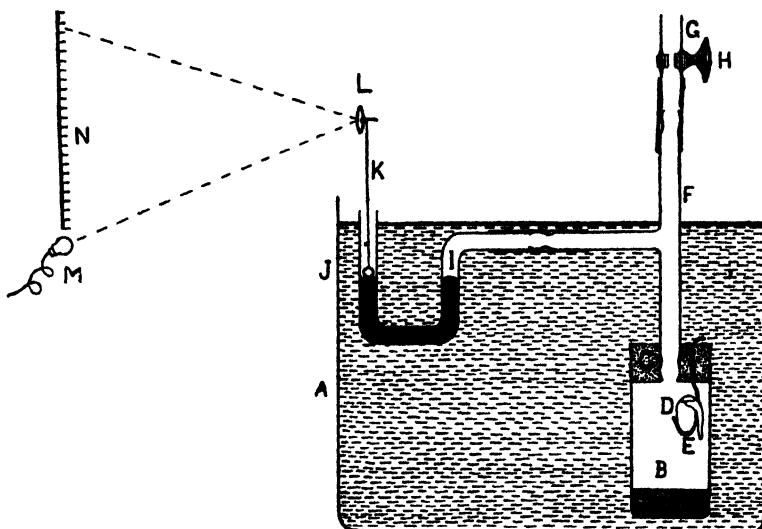
Fig. 1. Diagram of the micro-respirometer. A, Trough of water with a film of oil ; B, Specimen tube with caustic soda solution ; C, Rubber stopper ; D, Germinating seed ; E, Clamp ; F, 'T' tube; G, One-way glass tube ; H, Stop-cock ; I, 'U' tube with mercury ; J, Float ; K, Silk fibre ; L, Optical lever ; M, Light ; N, Scale.

the possibility of any fall in temperature due to evaporation of water. The tube (B) contains strong caustic soda solution at its bottom to absorb readily any carbon dioxide liberated during respiration. The germinating seed (D) is wrapped in moist blotting paper and fixed in the clamp (E). The wire from the clamp projects into the water outside. This is a device to remove immediately any heat evolved during respiration. The stopcock (H) allows or cuts off communication with the exterior, the latter being necessary before taking readings. During respiration the seed makes use of the oxygen in the chamber and liberates carbon dioxide. The ready absorption of the latter by caustic soda solution results in the rise of mercury in the closed end of the U-tube

(I). The float is attached to the short arm of the optical lever (L), the other arm being the beam of light. A galvanometric mirror mounted on the balance wheel of a watch and capable of revolving on the fine bearings forms the most important part of this optical lever. A stand with rack and pinion arrangement facilitates the proper adjustment of the optical lever and of the beam of light on the scale before recording is started. Any small depression of the float will result in the movement of the mirror in the clockwise direction, with the consequent upward movement of the focussed beam of light. Shifting the place of attachment of the float towards or away from the mirror results in a higher or lower magnification respectively. The author has made use of a mirror of 2 metre focal length, and the minimum distance at which the beam of light was focussed happens to be 2 metres. When very high magnification is necessary the distance between the scale and the mirror can be increased (this depending on the length of the room and the power of illumination) or the distance between the place of float attachment and the fulcrum reduced. By increasing the distance between the scale and the mirror to 3 metres and by reducing the distance between the fulcrum and the place of attachment to 2 mm. it is possible to have a magnification of 3,000. The movement of the beam of light projected on the scale (N) indicates the change in volume due to respiration of the seed. The shortest distance that could be observed without the help of a lens happens to be 1/40 inch. Since the diameter of the U-tube employed happens to be 4 mm. the movement of the beam of light through this distance will indicate a change in volume by nearly 1/384000 c.c., when the magnification happens to be 3,000. But for the present investigation the magnification employed was 400, thus making the smallest volume about 1/51200 c.c. for 1/40″ distance on the scale. While drawing the graphs the rates obtained from the movement of the beam at minute intervals have been doubled or quadrupled to enable a proper reproduction of the figures after reduction.

MATERIALS AND METHOD

The room temperature was constant during the brief period of observation in each case. The apparatus was tested before it was set up for observation. Control experiments were set up to detect the variation of pressure, if any, due to moisture. For this purpose a piece of wet blotting paper, instead of a germinating seed, was introduced into the clamp, and recording was started after an interval of an hour or more. The readings taken at minute intervals and with a very high magnification indicate that the pressure inside remains constant during a sufficiently long period. Care was taken to enable the chamber to attain saturation in humidity by introducing extra quantity of wet blotting paper for wrapping the seed. It may be stated that since the tube is a closed chamber there is every possibility of the air in the chamber reaching a stage of saturation in humidity since the quantity of

moisture lost from the blotting paper and from the seedling due to transpiration and respiration will be very much greater than the quantity of water absorbed by the surface of alkali solution during the same period. Depending on the quantity of water available in the blotting paper the stage of equilibrium in the saturation can be maintained for several hours at a stretch. Fresh solution of alkali was used for each experiment for the absorption of carbon dioxide as quickly as possible. Since the distance between the solution and the seed is very short the possibilities are in favour of small volumes of carbon dioxide settling on the solution with minimum delay, only to be readily absorbed by it.

Germinating seeds of *Dolichos lablab*, *Cicer arietinum*, *Phaseolus vulgaris*, *Pisum sativum* and *Zea Mays* were selected for studying the rate of respiration. The seed coat was carefully removed in all, except *Zea Mays*, and the germinating seed or young seedling was carefully washed and weighed before this was used for the experiments. The germinating seed was left in the apparatus for a period of about $\frac{1}{2}$ hour or more before recording was started. Necessary magnification was adjusted and the readings were taken at intervals of a minute for a period of $\frac{1}{2}$ to 1 hour or more. The particulars connected with the author's observations on the respiration of a germinating seed of each plant are presented in a tabular form, and the variation in the rate of respiration has been represented in the form of graphs given below.

OBSERVATIONS

The following tabular statements give an idea of the weight of the germinating seed, duration of experiment, magnification employed, volume of oxygen utilised, room temperature and other particulars.

The five graphs introduced in this paper show the rates of respiration in the germinating seeds of different plants. The figures 2, 3, 4, 5 and 6 are the graphs of the germinating seeds of *Dolichos lablab*, *Cicer* sp., *Phaseolus vulgaris*, *Pisum sativum* and *Zea Mays* respectively. From these figures and the data connected with each it is noticed that respiration does not go on at a uniform rate but is given to fluctuations in its rate from time to time. There are periods of high activity alternating with those of reduced ones, these resulting in the major fluctuations in the graphs. Respiration proceeds on at a high rate only for a few minutes, the period of high activity being generally between 6 to 10 minutes or more depending on the kind of young seedling. During the periods of major fluctuations there are minor fluctuations in the rate, these occurring at intervals of 1 to 2 minutes, or more. There is appreciable difference between the highest and lowest rates of this activity, the former being at times four to ten times the latter as is seen in the graphs.

Data connected with the respiration of a germinating seed of Dolichos lablab (Fig. 2)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes	0·80	0·80	0·70	0·65	0·50	0·65	0·70	0·55	0·65	0·60	6·60	76° F.
2nd ,,	0·60	0·70	0·65	0·90	0·90	0·75	0·90	0·95	...	0·75	7·20	,,
3rd ,,	1·05	1·00	0·75	0·85	0·60	0·85	0·75	0·80	1·00	1·10	8·75	,,
4th ,,	0·60	0·70	0·60	0·50	...	...	...	...	...	...	2·40	,,

Movement at the end of 33 minutes of activity .. 24·95

Weight of the germinating seed	.. :	0·812 gm.	Magnification employed	.. :	×400
Period of activity	.. :	33 minutes	Fall in the mercury column	.. :	0·156 cm.
Distance travelled by the beam	.. :	24·95 inches	Volume of oxygen absorbed	.. :	0·0195 c.c.

Data connected with the respiration of a germinating seed of Cicer arietinum (Fig. 3)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes	0·425	0·925	0·85	0·95	0·80	0·75	0·55	0·35	0·25	0·15	6·00	74·5° F.
2nd ,,	0·150	0·150	0·25	0·30	0·75	0·20	0·40	0·275	0·925	0·85	4·25	,,
3rd ,,	0·300	0·600	0·50	0·60	0·60	0·75	0·85	0·300	1·250	...	5·75	,,
4th ,,	...	0·400	0·10	0·20	0·30	0·25	0·15	0·200	0·200	0·15	1·95	,,
5th ,,	0·150	0·050	0·15	0·15	0·10	0·05	0·10	0·100	0·150	0·10	1·60	,,
6th ,,	0·100	0·150	0·05	0·15	0·15	0·15	0·15	0·200	0·250	0·25	1·60	,,
7th ,,	0·250	0·550	0·50	0·85	1·10	0·90	0·95	1·000	0·800	0·80	7·70	,,
8th ,,	0·900	0·900	0·85	0·90	...	...	...	...	...	...	3·55	,,

Movement at the end of 72 minutes of activity .. 31·90

Weight of the germinating seed	.. :	0·258 gm.	Magnification employed	.. :	×400
Period of activity	.. :	72 minutes	Fall in the column of mercury	.. :	0·199 cm.
Distance travelled by the beam	.. :	31·9 inches	Volume of oxygen absorbed	.. :	0·025 c.c.

Data connected with the respiration of a germinating seed of Phaseolus vulgaris (Fig. 4)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes	0·55	0·55	0·575	0·575	0·65	0·75	0·75	1·15	0·90	0·525	6·975	75° F.
2nd ,,	0·675	0·55	0·575	0·625	0·65	0·375	0·775	0·60	0·55		5·375	,,
3rd ,,	0·70	0·80	0·85	1·00	0·95	1·075	1·075	0·55	0·70	0·45	8·150	,,
4th ,,	0·55	0·50	0·325	0·25	0·325	0·650					2·600	,,

Movement at the end of 35 minutes of activity .. 23·100

Weight of the germinating seed	..	..	1·045 gm.	Magnification employed .. ×400
Period of activity	..	..	35 minutes	Fall in the column of mercury .. 0·144 cm.
Distance travelled by the beam	..	..	23·10 inches	Volume of oxygen absorbed .. 0·018 c.c.

Data connected with the respiration of a germinating seed of Pisum sativum (Fig. 5)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes	0·15	0·375	0·45	0·15	0·475	0·35	0·35	0·05	0·15	0·55	3·05	74° F.
2nd ,,	0·35	0·525	0·225	0·25	0·275	0·425	0·60	0·45	0·35	0·225	3·675	,,
3rd ,,	0·425	0·45	0·40	0·70	0·55	0·45	0·50	0·35	0·45	0·30	4·575	,,
4th ,,	0·50										0·50	,,

Movement at the end of 31 minutes of activity .. 11·80

Weight of the germinating seed	..	..	0·455 gm.	Magnification employed .. ×400
Period of activity	..	..	31 minutes	Fall in the column of mercury .. 0·074 cm.
Distance travelled by the beam	..	·..	11·80 inches	Volume of oxygen absorbed .. 0·0093 c.c.

Data connected with the respiration of a germinating seed of Zea Mays (Fig. 6)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes ..	0·625	0·575	0·75	0·575	0·70	0·575	0·75	0·875	0·875	0·825	7·125	74° F.
2nd ,, ..	0·875	0·85	0·85	1·00			0·575	0·70	0·625	0·725	6·20	,,
3rd ,, ..	0·60	0·95	0·675	0·725	0·575	0·95	0·575	0·50	0·50	0·60	6·65	,,
4th ,, ..	0·60	0·55	0·80		0·40	0·425	0·25	0·675	0·65	0·40	4·75	,,
5th ,, ..	0·55										0·55	,,

Movement at the end of 38 minutes of activity .. 25·275

Weight of the germinating seed .. .. : 0·275 gm.
Period of activity .. .. : 38 minutes
Distance travelled by the beam .. . 25·275 inches

Magnification employed .. : > 400
Fall in the column of mercury .. : 0·158 cm.
Volume of oxygen absorbed .. : 0·0198 c.c.
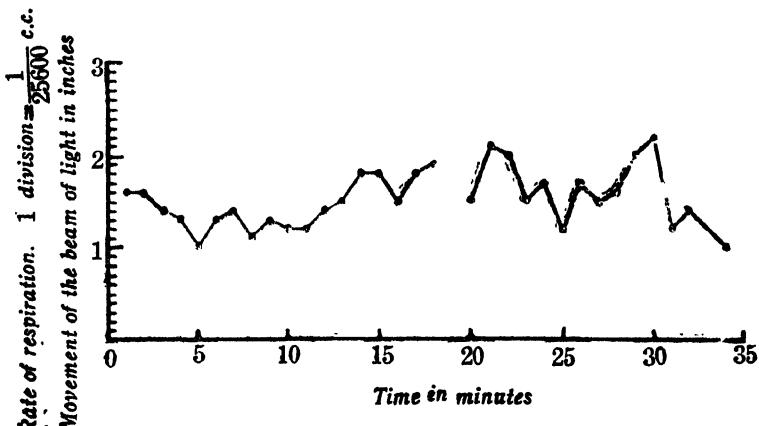
Fig. 2. Graph to show the variation in the rate of respiration in *Dolichos lablab.* ×2.
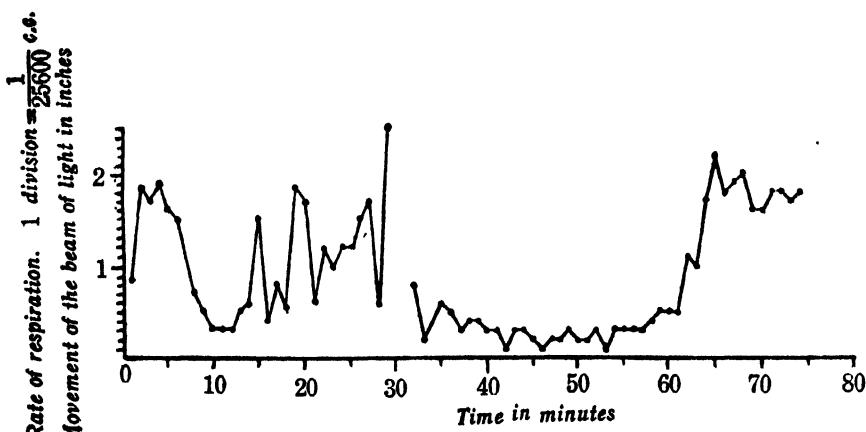
Fig. 3. Graph to show the variation in the rate of respiration in *Cicer.* ×2.
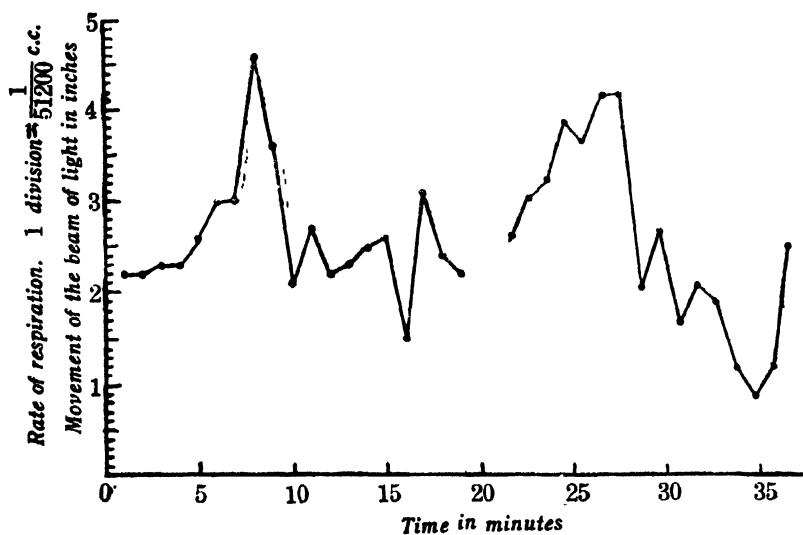
Fig. 4.   Graph to show the variation in the rate of respiration in *Phaseolus vulgaris.* ×4.
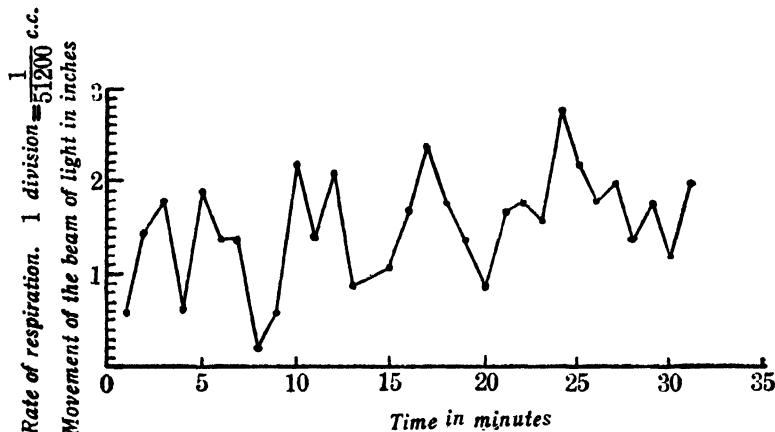
Fig. 5.   Graph to show the variation in the rate of respiration in *Pisum sativum.* ×4.
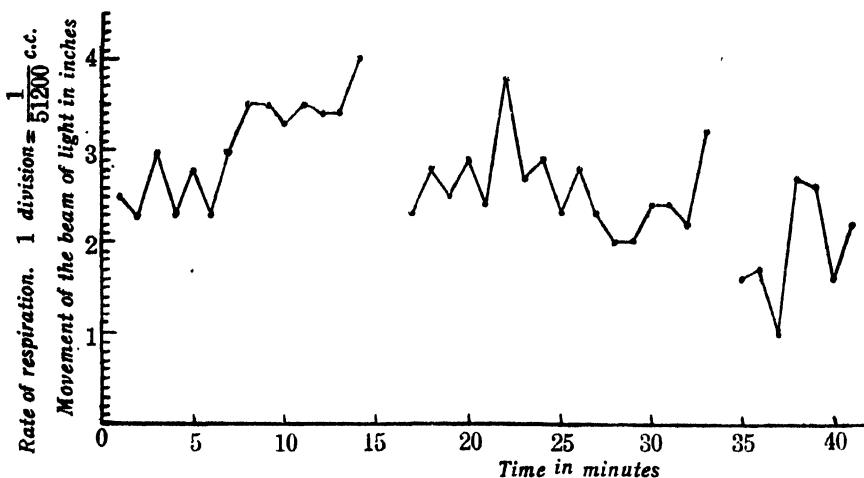
Fig. 6.   Graph to show the variation in the rate of respiration in *Zea Mays*.
×4.

DISCUSSION

From the above account it is found that notwithstanding constant external conditions there are momentary and periodical variations in the rate of respiration. A detailed enquiry into the external factors affecting respiration is out of place since the conditions are maintained almost constant during the brief periods of observation.

The experiments of Hopkins (1926) on potato illustrate not only the influence of temperature but also the effect of diastase and the accumulation of sugar on this activity. In the present case one is inclined to believe that a temporary or momentary and purely local variation in temperature within the tissues is a possibility and that this might have its own share in the variation of the activity of diastase and consequently momentary variation in the rate of respiration as shown by the minor fluctuations in the graph.

Role of moisture in respiration and its variation has been clearly explained by Bailey (1918) and other investigators. The author's observations on the leaf movements and water absorption (Krishna Iyengar, 1942 *b*) indicate that in many plants the water-content of the plant body will be varying even at short intervals of part of a minute. If similar fluctuations occur in the tissue of the germinating seed the possibilities are in favour of noticeable fluctuations in the rate of respiration also.

According to Miller (1931) the quantity of oxygen in the intercellular spaces of fruits and other plant parts primarily due

to poor gaseous exchange is often considerably below that of the air outside and may thus in some cases be limiting factor in the respiration of these parts. It is not improbable that momentary accumulation of carbon dioxide or the depletion of oxygen or both might temporarily affect respiration directly, and indirectly by affecting enzymatic activity resulting in the small oscillations at short intervals of a minute or less.

Finally the tone of the tissue may also count a great deal in deciding the rate of respiration; and its variation depends on several metabolic activities. The author's study of photosynthesis (Krishna Iyengar, 1942 *a*), leaf movement, water absorption and transpiration (Krishna Iyengar, 1942 *b*) and even growth (Krishna Iyengar, 1942 *c*) points towards the occurrence of variations in the rates of all these activities in several plants even when the external conditions are almost constant, indicating an oscillation in the rates of all these at short as well as at long intervals. Respiration shows similar fluctuations. In all these activities an active period will invariably be followed by a period of depression. These indicate the existence of possible fluctuation in the tone of the tissue from time to time, with its appreciable influence on the rates of all the vital activities. In conclusion it may be stated that while minor variations in the rate of respiration may be due to several factors like the temporary and purely local variation in the temperature due to respiration, fluctuations in the water-content, enzymatic activity and the available quantity of respirable material at a particular time and concentration of oxygen or carbon dioxide in the intercellular spaces, major variations which occur at intervals of 6 to 10 minutes or more can only be attributed to the possible fluctuations in the tone of the living tissue from time to time.

SUMMARY

1. The germinating seeds of *Dolichos lablab, Cicer arietinum, Phaseolus vulgaris, Pisum sativum* and *Zea Mays* were selected for studying respiration.

2. A special micro-respirometer was designed and the readings were taken at minute-intervals and the graphs were drawn.

3. A single germinating seed was taken at a time and the rate of respiration recorded.

4. The graphs represented indicate the occurrence of major and minor fluctuations in the rate of this activity, the former generally occurring at intervals of 6 to 10 minutes or more (depending on the nature of the seed, time of the day, kind of plant, internal activities, etc.), while the latter at intervals of a minute or two—at times even less than a minute.

5. Some of the factors like temporary or momentary and purely local variations in temperature due to respiration, fluctuating water-content, enzymatic activity, available quantity

of respirable material at a particular time and variation in the concentration of carbon dioxide and oxygen in the intercellular spaces of the tissue are probably responsible for the variation at short intervals.

6. The periodic variation in the tone of the living tissue is an important factor, and this seems to be reflected in the alternating periods of high activity and depression seen not only in respiration but also in several other vital activities of the plants.

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DIFFERENTIATION OF VASCULAR TISSUES IN *HIBISCUS SABDARIFFA* LINN.

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CONTENTS

INTRODUCTION

THE importance of the fibres of *Hibiscus sabdariffa* L., particularly the variety *altissima*, as a substitute for jute has been stressed by some scientists during the last few years. The present work forms a part of the studies undertaken at the Botanical Laboratory of the Presidency College, Calcutta, on the development and structure of fibres of this plant. This paper deals with only the differentiation of the protoxylem and protophloem ; but a detailed anatomy of the plant along with the development, structure and nature of fibres is being continued and the results will be published subsequently.

MATERIAL AND METHODS

The specimens were obtained from plants grown in the Gardens of the Presidency College, Calcutta, from seeds received through the courtesy of the Agricultural Department of the Government of Bengal.

Tips of vegetative shoots were killed in Formalin-Acetic-Alcohol after previous treatment with Carnoy's fluid and taken through grades of alcohol and xylol in the usual way. Sections were cut 8 to 10 μ thick and stained with Safranin and Light Green or Safranin and Fast Green. The cutting of sections was often facilitated by dipping the paraffin blocks in water for 12 to 24 hours.

Observations were also made from materials macerated in 5% chromic acid ; the macerated materials were stained with Safranin or aqueous Eosin and mounted in glycerine.

3

MORPHOLOGY

The plant is a small woody herb cultivated throughout the hotter parts of India and Ceylon. It completes its life-cycle from the germinating seed to death following fruit production within a single growing season. The stem yields a strong, silky fibre, the *Roselle hemp* of commerce, obtained by retting the stems when the plants are just in flower. It can grow in situations where jute cannot, and that is why it is believed by some that there are immense possibilities for this fibre as a substitute for jute in areas where the latter cannot be cultivated. Besides the fibre, other parts of the plant are also useful. The calyx of the flowers grows along with the fruits and becomes fleshy. It is a valuable antiscorbutic and is often eaten in the form of chutneys and jelleys. The seeds are reported to yield a kind of oil.

The plants attain a height of 8 to 10 feet and sometimes even 15 feet. The leaves are arranged spirally, the phyllotaxy varying from 2/5th in young shoots to 3/8th in vigorously growing shoots. The axillary buds produce branches which do not grow vigorously and as a result the general appearance of the plant is tall and erect.

SHOOT APEX

A series of microtome sections of an actively growing stem tip across the vegetative apical bud of a vigorously growing shoot showed 8 leaves crowded around the shoot apex, which are distinctly arranged in a 3/8th phyllotaxy (Fig. 1). There is no indication of the presence of an axillary bud in the axils of the first four primordia. An axillary bud develops in the axil of the 5th primordium. The development of the first internode is initiated between the 4th and 5th primordia.

The general manner of distribution and increase in the size of the leaf primordia in the shoot apex is given below in a tabulated series :—

Primordium	Level of insertion below the shoot apex*	Depth of insertion†	Length of free limb
	μ	μ	μ
1	10	20	50
2	20	30	290
3	60	60	770
4	140	130	1890
5	240	130	..
6	460	130	..
7	660	240	..
8	1060	..	..
9	1860	..	..

* Level of insertion is the point where the leaf primordium is becoming free from the stem.

† The region between the level of insertion of the primordium and the point where it is completely fused with the axis has been termed the " depth of insertion".

VASCULAR DIFFERENTIATION

Differentiation of vascular tissues is first noticed in the median bundle of the 3rd primordium which may be attributed to its more vigorous growth in comparison with that of primordium 2. The length of the free limb of primordium 3 was observed to be 770 μ while primordium 2 was only 290 μ in length. For this vigorous growth a greater food supply was required and as a result differentiation of vascular elements was found to take place in order to meet this demand. One protoxylem and one protophloem element was observed in this primordium (Figs. 1 and 2); the former differentiates as an isolated element at 120 μ below apex. From here the course of the xylem was traced in the free limb of this primordium and it was found to extend to a distance of 540 μ in the leaf while the phloem element continued still further up to a distance of 600 μ in this primordium. The lateral traces are also differentiated in primordium 3 but they remain in the procambial condition. The position of the protoxylem element is almost opposite to the sieve tube element of the protophloem which is also produced in the same desmogen strand.

The simultaneous differentiation of protoxylem and protophloem is contrary to the usual development of phloem before xylem, but it is not entirely unknown [see Kundu (1942) on jute].

At the insertion of primordium 3 there is no distinct vacuolation of the axis, but a continuous prodesmogen strand is found to have been developed. Vacuolation of the axis commences 100 μ below the growing tip, where only a few cells in the central region are found to have been vacuolated. The process of vacuolation is very rapid and only 60 μ below this point (*i.e.*, 160 μ below the growing tip) the central region (pith) is found to have been completely vacuolated. The process of vacuolation in cortical cells also begins simultaneously with those of the central region and at the level of 180 μ below the growing tip the central as well as the cortical regions are found to have been highly vacuolated.

At the insertion of primordium 4 most of the cells in the central region are found to have been vacuolated. At the point of fusion of primordium 4 with the axis, it is found that vacuolation has started even in the ray cells giving rise to the formation of 4 strands.

The primary rays are uniseriate and the procambial cells are in more or less radial alignment resembling those of jute (Kundu, 1942).

Protoxylem

The first xylem elements to appear are the small uninucleate spiral elements. In the procambial strand 120 μ below the apex, the first indication of protoxylem vessel was noticed. A few of the cells of the procambium were found to increase markedly in length and were seen to be associated with surrounding meristematic cells, which divide radially longitudinally and irregularly
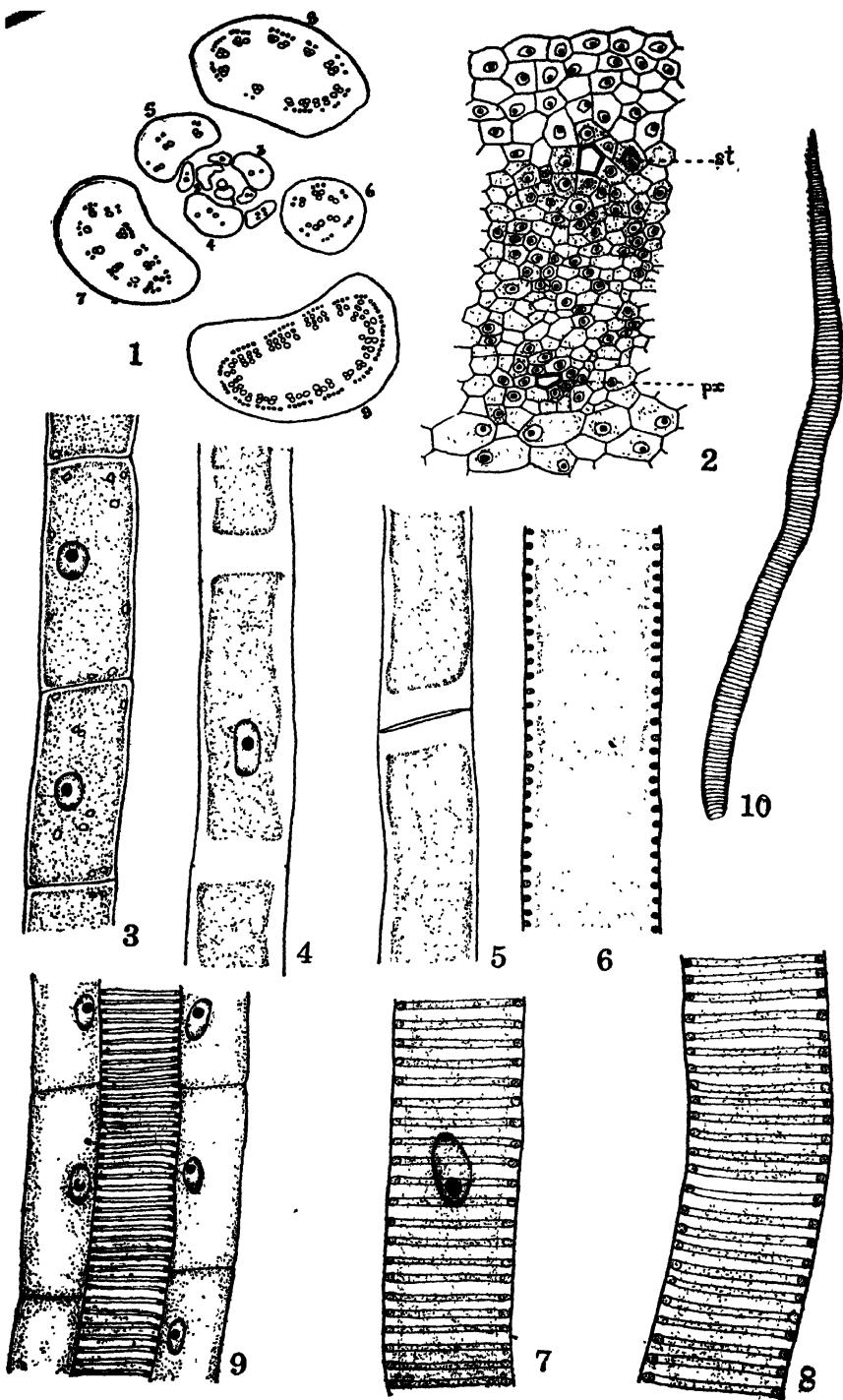
Text-figs. 1–10.—Fig. 1. Transverse section of the apex of a vigorously growing shoot (×40). Fig. 2. Transverse section of a portion of the 3rd primordium showing differentiation of a sieve tube and a protoxylem vessel. *st.*, sieve tube ; *px.*, a protoxylem vessel (×467). Fig. 3. Vessel mother cells in longitudinal section (×650). Fig. 4. File of protoplasts (×650). Fig. 5. Vessel segment with pectin film (×650). Fig. 6. Portion of a vessel segment showing deposition of bases and banding of cytoplasm. In macerated material and also in sections from fixed material the protoplasm appears to be contracted from the cell wall (×650). Fig. 7. Portion of a vessel segment with spiral secondary thickening and protoplasmic contents (×650). Fig. 8. Portion of a vessel segment with spiral thickening and degenerating cytoplasm (×650). Fig. 9. Vessel segment showing spatial adjustment of surrounding cells (×300). Fig. 10. An entire tracheid-like vessel segment at one end of which there is a perforation ; the other end is pointed and has no opening (×367).

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to give rise to xylem parenchyma cells. These cells are narrower than the vessel segments and are distinguished from the neighbouring pith cells by their size and protoplasmic contents. The developing vessel segments are in vertical series and they develop directly from the desmogen cells without further division.

A young vessel mother-cell is a growing meristematic cell full of contents (Fig. 3). A few such cells are arranged end to end ; during the earlier stages the end walls of such cells are clearly visible separating the young vessel elements. At the first stage of vessel differentiation the transverse end walls of the vessel mother-cells are dissolved and the protoplasm is seen to contract from the transverse and longitudinal walls. Though the end walls become dissolved the protoplasts of the developing vessel segments retain their individual characteristic and they form what is called the " file of protoplasts " (Fig. 4) (Priestley, Scott and Malins, 1935). This " file of protoplasts " is formed at a very early stage in vessel differentiation when the elements are extending and expanding and its existence is for a very short period. Soon after the formation of the file of protoplasts, a film of pectin is found to be developed at the position of the end wall in the form of a membrane (Fig. 5). In a longitudinal section this " pectin film " appears to be more or less lenticular in shape and limited above and below by two dark lines enclosing a light coloured substance. It disappears at a later stage, when the vessel segment has attained its maximum extension. Sometimes it exists even when deposition of thickening matters on longitudinal walls has started. While disappearing, the pectin films first appear as faint threads ; this thinning down of the pectin film indicates that it will soon be dissolved.

The first indication of secondary thickening is noticed in the deposition of bases on the inside walls of the vessel segments, when they have expanded fully (Fig. 6). These bases are thickenings on primary wall of vessel segments and they form localised projections into the cavity of vessel segments. On these projections thickening matters are laid. The presence of these projections on the wall of the vessel segment prevents the thickening matters to come into close contact with the wall. The bases appear as

continuous lines running along the centre of the thickening bands. They are not perceptible in highly lignified bands.

The protoplasm in the vessel segment suffers certain changes during the formation of the vessel. In a young cell it is uniform and dense, but as the cell increases in volume, the protoplasm contracts from the longitudinal walls as well as from the transverse end walls and fine vacuoles appear in it. Later on, with the formation of file of protoplasts these small vacuoles coalesce to form large vacuoles. In *Hibiscus* the banding of cytoplasm in finely and coarsely vacuolated regions at an early stage of vessel differentiation has not been observed as found by Barkley (1927) in *Trichosanthes* at a very high magnification. Nevertheless, when bases are deposited along the entire wall of the vessel segment the protoplast appears to be banded into thick and thin regions (Fig. 6). During all the stages of vessel differentiation and deposition of secondary thickenings the protoplast persists and the nucleus occupies central position of a vessel segment (Fig. 7). The nucleus degenerates shortly after the deposition of cellulose spiral band, but the cytoplasm persists upto the earlier stages of lignification in a fully developed vessel (Fig. 8).

It has been stated that protoplast appears to be banded after the deposition of bases. Along the thickened regions of the protoplast, deposits are formed on the walls over the bases and gradually they thicken and appear as rods. Staining reactions show that these bands are composed of pecto-cellulose but later on they change their chemical nature and become lignified.

The vascular elements of the protoxylem region sometimes have pointed end walls and as a result they appear somewhat like tracheids. In Fig. 10 is shown a vessel segment at one end of which there is perforation, the other end is pointed and has no opening.

Spatial adjustment.—During the differentiation of the vessels, the vessel segments exert great pressure on the surrounding cells which are mostly meristematic. As a result, these cells undergo rapid divisions radially longitudinally, transversely or sometimes irregularly. Thus, when the vessel segment is elongating, spatial adjustment is brought about by a rapid division of the surrounding parenchyma cells (Fig. 9).

Protophloem

The ontogeny of primary sieve tubes in *Hibiscus* has been studied from transverse as well as from longitudinal sections. The development of the protophloem strand begins with the differentiation of the first sieve tube at the outer periphery of the median desmogen strand of primordium 3 (Fig. 2). In transverse sections a protophloem sieve tube can be recognised by its swollen wall which stains markedly with Light Green or Fast Green. If this element is followed downwards it is found that it soon becomes crushed and degenerated. Thus if we follow from the apex we find that a protophloem element develops from a procambial cell

and this element becomes crushed downwards due to the continued pull and pressure exerted by surrounding tissues. Hence evidently, primary sieve tubes begin to arise acropetally from those formed earlier, while the older ones are continually crushed and become obliterated.
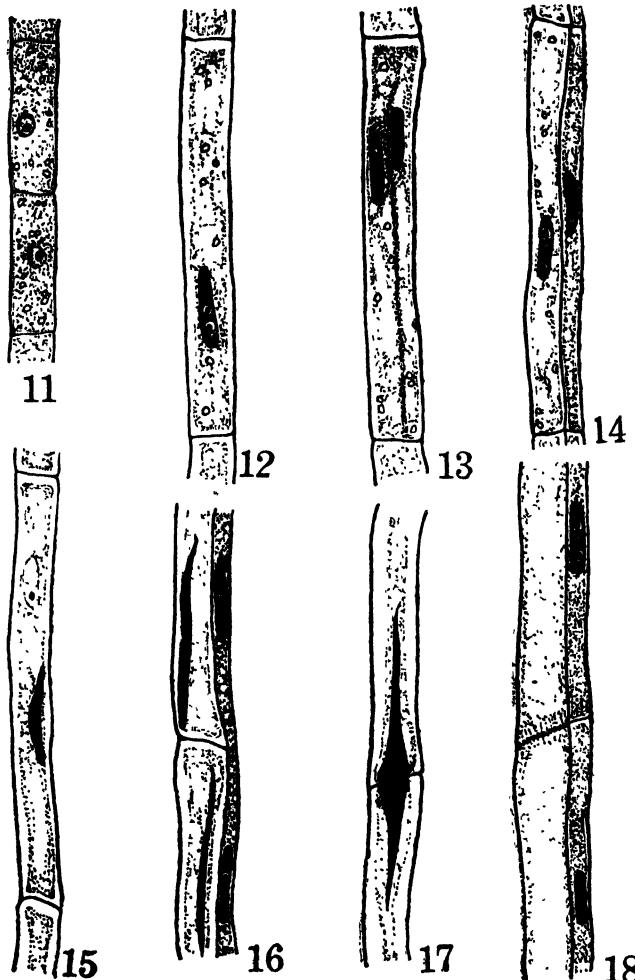
Text-figs. 11–18.—Fig. 11. Phloem mother-cells ($\times 975$). Fig. 12. An enlarging phloem mother-cell ($\times 975$). Fig. 13. Stage showing the cutting of a companion cell ($\times 975$). Fig. 14. A young sieve tube segment with a companion cell ($\times 975$). Fig. 15. Sieve tube segment with slime body and degenerating nucleus ($\times 975$). Fig. 16. Sieve tube segments with elongated slime bodies ($\times 975$). Fig. 17. Sieve tube segments with slime plug ($\times 975$). Fig. 18. A mature sieve tube with a companion cell ($\times 975$).

The sieve tubes have one companion cell each. Phloem parenchyma consists of somewhat elongated cells with transverse walls. The companion cells are not easily recognised in transverse sections in very young regions because many of the procambium cells are similar in size to the sieve tubes and companion cells and have as dense protoplasts as the companion cells.

The development of a sieve tube from its earlier stages can best be followed from a study of longitudinal sections as well as from macerated material. A young protophloem element (a sieve tube mother-cell) is an elongated cell with heavy stained nucleus, uniform cytoplasm, and transverse end walls (Fig. 11). The nucleus contains one or more nucleoli. There are also several other small spherical particles in the mass of the protoplast and they are the characteristic sieve tube plastids.

In the next stage the sieve tube mother-cell is found to extend and the nucleus also becomes elongated and 2 or 3 nucleoli are found to be present in it. The cytoplasm also becomes less dense and vacuolated (Fig. 12).

Later on the nucleus divides with the formation of two nuclei of the same size and a wall is formed dividing the cell longitudinally into two each containing a single nucleus. One of these cells forms the sieve tube element and the other the companion cell. The young sieve tube is larger than the companion cell. The companion cells can also be differentiated from the sieve tubes by their dense protoplasts and prominent nuclei (Figs. 13 and 14).

After the companion cell has been cut off the cytoplasm of the sieve tube element becomes highly vacuolated ; its nucleus loses its chromaticity, swells up and degenerates gradually. At the time the nucleus degenerates, one or more characteristic slime bodies appear in the mass of cytoplasm (Fig. 15). In the early stage they are spindle-shaped, but later on tape like slime bodies have been found in the sieve tube element (Fig. 16). The slime bodies of two adjoining sieve tube elements usually come in contact with the wall separating the two elements and form what are called the " slime plugs " (Fig. 17).

When slime plugs are formed the protoplast of the sieve tube elements contract from the longitudinal walls which become thickened. This thickening of the walls is first noticed at the stage when slime bodies appear after the cutting off of companion cell and it reaches its maximum prominence in the latest stage of development of the element. The thickened walls appear glistening and pearly in transverse sections and have been termed " nacree " walls (Leger, 1897) (Fig. 2).

After the walls have thickened, the slime plug degenerates, the end wall develops very fine perforations and the cytoplasm forms a very thin parietal layer with network of very fine strands traversing the lumen.

DISCUSSION

Literature on the differentiation of vessels in angiosperms is scanty and the descriptions somewhat incomplete and contradictory. It is well known that the differentiation of vascular tissues is intimately connected with the development of leaves and the first differentiated vascular elements of the stem appear in localised regions which constitute the leaf traces.

In dicotyledons the differentiation of vessels usually commences in the primordium at its level of insertion on the axis and from there progresses downwards into the stem and upwards towards the apex of the primordium. This has been observed by a number of observers (Trecul, 1881, 1891 ; Weiss, 1883 ; De Bary, 1884 ; Priestley and Swingle, 1929 ; and other recent workers) and is now regarded as well established. Our observation is also in agreement with the above fact.

Regarding the relative time of appearance of the first phloem and first xylem elements, Sanio (1863) reported that in stems phloem elements appear before xylem. Russow (1872) and Leger (1897) also found the appearance of phloem before xylem ; they considered it to be a normal phenomenon in the differentiation of vascular elements in Phanerogams and Cryptogams. Chang (1935) reported that the development of the vascular tissue in a primordium begins with the differentiation of a sieve tube. But in *Hibiscus* it is found that protoxylem and protophloem elements differentiate at the same time and this condition is in agreement with the observations of Kundu (1942) on jute.

According to most authors the cells destined to form the protoxylem vessels are uninucleate but F. M. Scott (1937) asserts that in *Ricinus* they are uninucleate in the younger internodes but multinucleate in the older ones. In *Hibiscus* there is no such coenocytic stage and the nucleus occupies a central or more or less central position in the vessel segment until its disintegration at the maturity of the vessel.

Regarding the breaking down of the end wall there is a great difference of opinion. According to Eames and MacDaniels (1925) the vessel segments reach their full size and permanent shape with the end walls unperforated. Esau (1936) found that in Celery the end walls break down after the maturation of the secondary wall. Barkley (1927) also refers to the late breaking down of end walls. F. M. Scott (1937) on the other hand observed that the end walls disappear in the coenocytic stage. According to Priestley and his co-workers (1935, 1938) the end walls of a vessel segment disappear at a very early stage even before the segments have attained their maximum extension. Sometimes the transverse wall splits in the middle, contracts and sticks to the sides of the vessel segments thus forming what is called the *rim*. Though the wall dissolves the protoplasts of different vessel segments do not come in contact but retain their individual characters and form what is known as " file of protoplasts ". Our observations on *Hibiscus*

agree with those of Priestley, Scott and Malins (1935) and Majumdar (1940) and the "file of protoplasts" develops at a very early stage of the differentiation of the vessels. The pectin film, which becomes deposited in the position of the dissolved end wall soon after the formation of the file of protoplasts appears lenticular in shape and looks like a thickened end wall as described by Esau (1936.)

Regarding the manner of disappearance of the end walls Esau regards it as a process of dissolution. Eames and MacDaniels also describe it similarly and in *Robinia* suggested an association of the nucleus with the formation of the vessel pores. In *Hibiscus* the end walls of the developing vessel segments disappear very early, long before they attain their maximum extention. The manner of disappearance of the end walls could not be followed thoroughly. It must be an abnormally rapid process as in none of the prepared slides intermediate stages of the breaking up or the dissolution of the end walls could be traced. But it seems to us to be a process of dissolution; in no case, however, was the nucleus found to be associated with the disappearance of end walls, as its position remains unchanged and it lies at or near the centre of vessel segment until its disintegration at the maturity of the vessel. In *Hibiscus* the pectin film which is formed later and resembles the end wall described by Esau (1936), does not dissolve before the maturation of the secondary wall; sometimes, however, it disappears before the deposition of thickening matters. The nucleus does not take any part in the disappearance of the pectin film. The part played by the pectin film in the development of vessels is not quite clear.

The early stages of secondary thickening and the progress of lignification takes place while the protoplast still persists. Barkley (1927) has pointed out that before secondary thickening the protoplast is banded into alternate finely and coarsely vacuolated regions. In *Hibiscus* banding of cytoplasm has been observed simultaneously with deposition of bases. The deposition of bases before an actual secondary thickening of the vessels seems to be a special feature. This phenomenon was first noticed by Rothert (1899) (cited in Haberlandt, 1914). Eames and MacDaniels (1925), Barkley (1927), Esau (1936) and Scott (1937) did not mention anything about the deposition of bases. Recently Majumdar (1940) has observed them. The bases are formed in earlier stages and appear in the pattern of secondary thickenings on the walls. On these bases cellulose bands are deposited. The bands later on become lignified, and the protoplast of the vessel segment persists till lignification is complete.

As regards the manner of spatial adjustment during differentiation of vessels, Eames and MacDaniels state that no cells are broken during adjustment. Krabbe (1886) noticed tearing apart of adjacent cells during development of vessels and this was confirmed by Priestley, Scott and Malins (1935) in the expansion

of secondary xylem vessels. Esau (1936) has also pointed to the same method of adjustment. In *Hibiscus* spatial adjustment is brought about by active radial, longitudinal, transverse or irregular division of the surrounding cells and this agrees with the observations of Majumdar (1940) in *Heracleum*.

The failure of some workers (Eames and MacDaniels, 1925) to recognise sieve tubes in the protophloem may perhaps be attributed to their comparatively indistinct sieve plates. Nevertheless, existence of sieve tubes in the protophloem was reported by many early workers like Russow (1872) (originator of the term protophloem), De Bary (1884), Lesage (1891), Leger (1895, 1897) and Chauveaud (1897, 1900) and more recently by Chang (1935) and Esau (1934, 1935, 1936, 1938).

As regards the course of development of the protophloem, Baranetzky (1900) held that the protophloem differentiation went on simultaneously over the entire extent of the leaf trace. Griffiths and Malins (1930) have, however, observed that the protophloem elements differentiate in continuity with the phloem of the older parts of the plant, and that the first sieve tube of a particular leaf trace proceeds acropetally from the stem towards the leaf. These views have been confirmed by Chang (1935), Esau (1938) and Priestley and Scott (1938). The observations of the present writers are also in agreement with these.

The appearance of slime bodies during the development of the sieve tube has been recorded by several workers. Crafts (1933, 1934) reported that in tobacco they occur only in the primary sieve tubes. These slime bodies are generally characteristic of plants belonging to a certain family. The slime bodies in Solanaceæ have been found to be of the same nature by Kotila and Coons (1923), Doolittle and McKinney (1923), Kofoid and others (1923), Artschwager (1924), and Crafts (1933, 1934). Slime drops have been observed in the Cucurbitaceæ by Wilhelm (1880), Fischer (1886), Le Comte (1889) and Crafts (1932); and spindle-shaped slime bodies have been observed in Leguminosæ by Strasburger (1891), Baccarini (1892), Staritz (1893), Doolittle and McKinney (1923) and Bailey (1923). Some plants show no constancy of shape of the slime bodies, as in *Vitis* (Wilhelm, 1880). In *Hibiscus*, slime bodies were found to be spindle-shaped and occasionally tape-like in structure.

The nature of the slime bodies is proteinaceous and it is in agreement with the observations of several workers. Fischer (1885, 1886) reported that slime bodies become dissolved in the sieve tube sap in mature elements and according to Le Comte (1889) they disappear from the sieve tube cytoplasm by passing into the vacuole making the contents more viscous. The disappearance of slime bodies generally agrees in time with disintegration of nucleus but our observation shows that they persist even after the degeneration of the nucleus as found in the Leguminosæ by Strasburger (1890).

The majority of workers agree that the mature sieve tube does not possess any nucleus and that the sieve tube nucleus disappears as a discrete body (Wilhelm, 1880), Janczewski (1881, 1882), Schmidt (1882), Russow (1882), Strasburger (1882, 1887, 1891), Artschwager (1924), Crafts (1932, 1933, 1934), Esau (1934, 1935, 1936, 1938). Certain others report that they have seen nuclei even in the mature sieve tubes of some plants (Fischer, 1886 ; Le Comte, 1889 ; Schmidt, 1917). In *Hibiscus*, the nucleus has been found to disappear at an early stage when the slime bodies are appearing.

The sieve tube leucoplasts can be seen during the first stages of specialization of a sieve tube (Briosi, 1873 ; Wilhelm, 1880 ; Fischer, 1886 ; Crafts, 1934 ; Esau, 1934, 1935, 1936) and this corresponds with our observation on *Hibiscus*. The leucoplasts arise in the cytoplasm of young sieve tubes but may enter the vacuole in mature elements (Fischer, 1886 ; Crafts, 1933, 1934) and they are sometimes retained by the sieve tube until the elements begin to collapse.

SUMMARY

1. Differentiation of protoxylem and protophloem elements takes place simultaneously in the median desmogen strand of the third leaf primordium. Vacuolation of the axial cells starts 100 μ below the growing tip and 60 μ further below it is found to be completely vacuolated.

2. The first protoxylem elements which differentiate from the desmogen are small rectangular cells (vessel mother-cells). During the first stage of vessel differentiation the longitudinal walls of the enlarging vessel mother-cells remain thin but the transverse walls disappear. The protoplasts of contiguous vessel segments do not mix but retain their individual characteristics and form " file of protoplasts ". After formation of the file of protoplasts, a " pectin film " appears in the region of the transverse wall in the form of a membrane and separates the two developing vessel segments. This film which appears like an end wall is dissolved at a later stage in the development of the vessel.

3. During the process of secondary thickening of vessel segments, bases are laid down on their longitudinal walls in the pattern of the thickening matter to be deposited ; on these bases cellulose bands are deposited and these bands later become lignified.

4. The protophloem element (the sieve tube mother-cell) is an elongated cell having dense protoplasm and a number of spherical granules (the sieve tube plastids). This cell rapidly extends and by its longitudinal division a companion cell is cut off. Afterwards the nucleus of the sieve tube element gradually degenerates and one or more spindle-shaped or tape-like slime bodies appear in it. At this time the longitudinal wall of the sieve tube becomes thickened and the end walls develop fine perforations. The slime bodies degenerate and the cytoplasm forms a thin

parietal layer with network of very fine strands traversing the lumen.

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MORE ABOUT *HEMILEIA CANTHII*

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In a paper on *Hemileia Canthii* Berk. and Broome, published by M. J. Thirumalachar in the last issue of this *Journal* (Vol. XXII, Nos. 2, 3 and 4 of July 1943) it has been mentioned that this rust was collected in India on two hosts, *viz.*, *Plectronia parviflora* Bedd. and *Plectronia Rheedii* Bedd., at Yelwal, Mysore, and at Belgaum respectively.

In this connection it will be interesting to record here that the author of this note has observed the occurrence of *Hemileia Canthii* Bedd. on *Randia dumetorum* Lam. at Matheran in December 1927, and on *Plectronia Rheedii* Bedd., at Amboli Hills in January 1938, in the uredo and teleuto stages in the first case and uredo only in the second case.

For some reasons, this observation remained unpublished so far. In a recent visit (20th Dec. 1943) to Dajipore forests (Fonda ghauts), this rust was observed in the uredo stage only on the leaves of *Plectronia Rheedii* Bedd. It is not reported so far from this side.

THE BUFFALO-HORN BAMBOO OF BURMA

An Inadequately Known Species of Giant Bamboo

BY F. G. DICKASON

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(Communicated by Dr. R. R. Stewart, PH.D.)

Sinocalamus latiflorus (Munro) McClure has apparently been described only from herbarium material as the description is incomplete and taxonomically inadequate, many diagnostic characteristics not having been mentioned. Among these are some distinctive characteristics enabling field identification of the species in a vegetative condition. These are particularly important because so many species of bamboo can only be identified from the flower.

Sinocalamus latiflorus (Munro) McClure

1868   *Dendrocalamus latiflorus* Munro, *Trans. Linn. Soc.,* Vol. 26, p. 152.

1873   *Bambusa latiflora* Kurz, *Journ. As. Soc. Bengal,* Vol. 42, p. 250.

1896   *Dendrocalamus latiflorus* Munro, *Gamb. Bamb. Brit. Ind.,* p. 131.

1897   *Dendrocalamus latiflorus* Munro, Hooker, *Fl. Brit. Ind.,* Vol. 7, p. 407.

1913   *Dendrocalamus latiflorus* Munro, Camus, *Les Bambusees,* p. 160.

1921   *Dendrocalamus latiflorus* Munro, Brandis, *Ind. Trees,* p. 678.

1940   *Sinocalamus latiflorus* (Munro) McClure, in New Genera and Species of Bambusaceæ from Eastern Asia.

Material Examined

35 clumps in the field in the neighbourhood of Taunggyi in the Southern Shan States, Burma. Herbarium specimens, Judson College, Rangoon, Nos. 8288, 8372, 8678, 9397, 9398, 9399.

Description

Vegetative Characteristics.—The clumps of this species are from 80 to 100 feet high and rather open. The base of each culm leans out before turning erect, making a curve at the base of each culm like that of a water buffalo's horn (Fig. 2). The openness of the clump is accentuated by this peculiar way the culms have of leaning out before straightening up. No lateral branches are formed on new culms before April of the year following the rainy season (June to September) when the new shoots appeared. The lower 10–20 feet of the culm normally never develop lateral branches. The culms, when young, are covered with a grey-white bloom which is denser below the nodes than above. Mature stems are tough and hard, and are the ones most commonly used near Taunggyi for the posts of bamboo houses. The length of the internode 4½ feet above the ground varies from 8½ to 13 inches, and its circumference from 9½ to 17 inches. The average circumference/length ratio* of the internode at 4½ feet is $\frac{12 \cdot 25}{10 \cdot 80}$ in. or $1 \cdot 14$.

The culm sheath (Fig. 1) is stiff and hard, is highly polished inside, and has appressed brown hairs outside. At the lower nodes at least, the sheaths are longer than the internodes. These lower sheaths are about half again as broad as they are long, *i.e.*, 13 inches long by 22 inches broad. There are broad shoulders on the sheath, above which the sheath is truncate. There are no auricles or oral setæ. The ligule is about 5 inches broad and ¼ inch deep, the margin being serrate. The sheath blade (of a sheath at about 4½ feet above ground) is erect and is 2½ inches broad by 3¾ inches long. The ligule extends 1 or 1¼ inches on either side of the base of the blade which is slightly puckered before joining the sheath. There is a very distinct line separating the sheath from the blade.

Reproductive Characteristics.—When flowering occurs, the culms become great panicles with flexible, pendulous, lateral branches on which the reddish spikelets are arranged in heads at the nodes. The central lateral branch at each node is normally unbranched and on it the spikelets are in large heads of 60 or more ; the internodes of this branch vary in length from 4 inches near the main culm to less than 1 inch towards its apex. All other lateral branches than the central one branch repeatedly, and on them the spikelets are arranged in smaller heads which are almost confluent (Fig. 1). These two types of inflorescence branches are so different that they might easily be ascribed to different species if seen separately.

The lemmas and palets of the florets are very pubescent and long-ciliate on margins and keels. There are no veins between the keels of the lower palets but there are as many as two between the keels of the upper ones. The apex of the anther is pointed and bristly. The long cells of the inner layer of the anther wall are peculiar in containing many annular rings of thickening.

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* "The Circumference-Length Ratio," *Jour. Ind. Bot. Soc.*, 21, Nos. 5-6, 351-53
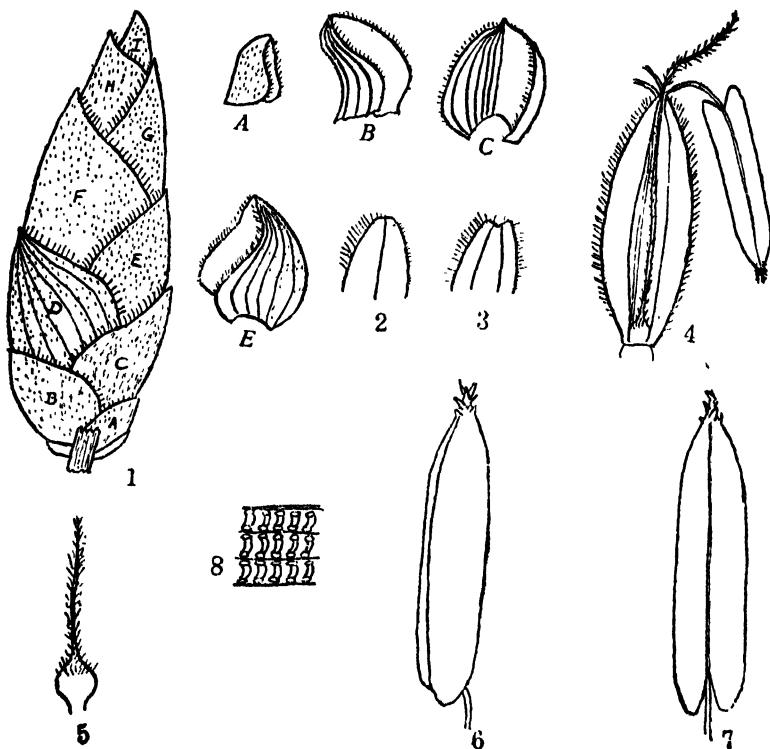
Figs. 1—8.—*Sinocalamus latiflorus* (Mun.) McClure. Fig. 1. Spikelet, 9/16 in. long, ¼ in. broad, 5/32 in. wide. A, B and C, empty glumes ; D, E, F, G, H and I, lemmas of florets. Oldest floret below, youngest above. Reddish brown in colour, shortly hairy, ciliate on margins. A—Lowest empty glume, 1/8 in. long. B—Second empty glume, 3/16 in. long. C—Third empty glume, 3/16 in. long. E—Lemma of second floret, 3/8 in. long. Fig. 2. Tip of palet of E, F or G, somewhat truncate, keels long ciliate, short hairy outside, with one vein between keels. The palet of the lowest floret with acute apex and no vein between the keels. Fig. 3. Tip of palet of H or I. Very slightly bidentate, 2 veins between keels, and 1 vein in each flap. Fig. 4. Palet of D, 5/16 in. long, showing style and anther exserted. Fig. 5. Pistil : ovulary hairy on upper half, style 1, hairy. Fig. 6. Anther, yellow, side view showing bristly acuminate apex. Fig. 7. Anther, front view, 7/32 in. long. Fig. 8. Part of inner wall of anther showing peculiar annular thickening.

Records of Flowering

Sinocalamus latiflorus (Munro) McClure flowered about Taunggyi in scattered clumps during the dry seasons of 1939 to 1941, anthesis beginning during the cold season. At no place did there appear to be general flowering, although nine miles east of Taunggyi at Hopone there were half a dozen clumps flowering within a stone's throw of each other.

Vernacular Names

In the Shan villages, particularly those surrounding Taunggyi, where *Sinocalamus latiflorus* is commonly cultivated, it is called in Shan, *Mai kao quai*, which means Buffalo-horn bamboo. This vernacular name refers to the shape of the young culm shoots and to the bases of the mature culms which slope outward and then up with the same curve as that of a water buffalo's horn (Fig. 2). Other Shan names used for this bamboo are *Mai pok leng* or *Mai leng* which means Red bamboo, probably in reference to the reddish brown spikelets of the inflorescence. The same characteristic has given rise to the name *Wa ni* (Burmese) which was reported from Maymyo in 1896. The Burmese name for this species at Taunggyi is *Wa gyi*, Big bamboo ; this name, however, is used for other species of bamboo and so is not distinctive. The name, Buffalo-horn bamboo, is preferable to the names based on colour inasmuch as it refers to a distinctive vegetative characteristic which is always present.

Remarks

This species is one of the two or three kinds of giant bamboo commonly cultivated in the Shan States. The outward slant and upward curve of the culm bases of this bamboo at once set it apart in the field from the other kinds with which it commonly grows. Should a clump be found where this characteristic is not well developed, then the clear line separating the culm sheath from its blade is sufficient to distinguish this species from other large types growing in the Southern Shan States.

This Buffalo-horn bamboo may be distinguished in a number of ways from a very similar bamboo, *Mai pok mon* (Shan) (probably *Bambusa Copelandi* Gamble) which appears to be widely cultivated in the Shan States, as may be seen from the following chart :

	Mai kao quai	*Mai pok mon*
Height	.. 80–100 ft.	.. Slightly shorter
Base of culm	.. Curves out and then up	. Lacks outward curve
Bloom on culm	.. Grey bloom denser below node than above	Grey bloom even throughout internodes
$\dfrac{\text{Circumference}}{\text{length}}$ ratio	.. $\dfrac{12.75 \text{ in.}}{11.30 \text{ in.}} = 1.13$	.. $\dfrac{13 \text{ in.}}{12 \text{ in}} = 1.08.$
Uses	.. House Posts	.. Matting
Culm sheath	.. Broad shoulders and truncate across top	Curved from base rather directly to base of blade
Ligule	.. 5 inches broad	.. 3 inches broad
Culm sheath blade	.. Erect A distinct line separates blade from sheath	.. Reflexed, at least by April. No distinct line : the tissues seem continuous
Inflorescence	.. Branches drooping, pendulous	.. Branches rigid, ascending
Spikelets	. Reddish-brown, Up to 60 or more in a head	Straw coloured, Up to 6 in a head

Fig. 1. (*Above*) Culm sheath from the node of *Sinocalamus latiflorus* 4½ feet above the ground, together with branches of the inflorescence, the central undivided branch being shown on the left.

Fig. 2. (*Below*). Base of a clump of *Sinocalamus latiflorus* which is in flower, showing the outward slant of the culms and the central undivided lateral branches of the inflorescence.

F. G. DICKASON--

THE BUFFALO-HORN BAMBOO OF BURMA

SUMMARY

In this paper *Sinocalamus latiflorus* (Munro) McClure of the Shan States, Burma, has for the first time been definitely linked with a Shan name, *Mai kao quai*. Additional characteristics, both vegetative and reproductive, are given which have not previously been described. A comparative chart has been worked out showing how this species differs from another similar giant bamboo, *Mai pok mon*, commonly cultivated in the same region.

VARIATION OF LEAF-FORM IN
POTAMOGETON PERFOLIATUS L.

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AN opportunity to study the causes of form-variation in *Potamogeton perfoliatus* was afforded while studying the ecology of water plants in the English Lakes. Many other pond-weeds such as *P. praelongus*, *P. alpinus*, etc., also exhibit variations in the shape and size of their leaves in different lakes and even in the same lake at different places. Nevertheless, the variations in form are far more pronounced in *P. perfoliatus* than in these other species. Taxonomists usually split the natural forms into various sub-species on account of this feature (*cf.* Hagstrom, 1916). However, Fryer, Bennett and Morgan (1915) think that all the British forms of the species may possibly be mere states and not varieties. It was thought that the habitat might be partly responsible for the variations in the species and hence this study was undertaken as an aid to ecological study of the aquatic plants now published elsewhere (Misra, 1938).

 P. perfoliatus is a characteristic species of silted zones in the lakes where organic matter decomposes readily. In shallower water, it grows only in land-locked bays but in deep water it can grow in exposed parts upto a depth of six metres. The plants growing in Lake Coniston have usually long internodes and narrowest leaves which are thin and olive green in colour. On the other hand, the plants of Ullswater and of the calcareous river Wharfe possess short internodes and broadest leaves which are usually thick, pale coloured and with well-developed veins. They are also usually larger in size than any other forms. The species collected from the rest of the lakes vary between these two extreme forms which differ from one another much as do shade and sun leaves. But, although the plants growing in deeper parts of a lake usually possess somewhat longer internodes yet there is apparently no correlation between depth and shape of the leaf. Thus light intensity as judged by the depth of the water has no obvious influence upon the shape of the leaf. Movement of water is also not responsible for the variations as the river forms are identical with many of the lake forms.

SHAPE VARIATIONS IN ADULT LEAVES

 A fully grown plant shows slightly broader leaves at the base and the top ends of the main axis than the leaves present at its middle part. The same sequence of leaf shape is found upon the individual branches although the leaves are usually much smaller in size. Since changes in the form of the leaves are mainly due to the relative variations of length and breadth the shape of the leaf can be expressed

numerically by the ratio $\dfrac{\text{length}}{\text{breadth}}$ (or briefly, L/B) as suggested by Pearsall and Hanby (1925). The statistics of this ratio obtained from different localities are given in Table I. The mean L/B ratios of leaves borne on different plant organs are tabulated with the standard error along with the standard deviation with its error and the variance.

TABLE I

Statistics of L/B ratios

Locality	Shoot parts	No. of leaves measured	Mean	Standard deviation	Variance
1. River Wharfe	Main axis	229	2·000 ±0·019	0·283 ±0·013	0·080
2. Lake Ullswater	Main axis	52	2·920	..	..
	Branch	35	2·810	..	..
	Flowering shoot	8	2·310	..	.
3. Lake Coniston	Main axis	156	3·776 ±0·068	0·851 ±0·048	0·724
	Branch	38	3·579 ±0·147	0·903 ±0·114	0·816
	Flowering shoot	11	2·750 ±0·091	0·301 ±0·064	0·099
4. Lake Windermere—					
(a) Low Wray Bay	Main axis	171	3·654 ±0·019	0·346 ±0·027	0·120
	Branch	114	3·877 ±0·023	0·877 ±0·032	0·770
(b) Boat house (Wray Castle)	Main axis	178	3·960 ±0·012	0·154 ±0·010	0·237
(c) Sawpit Bay	Main axis	115	3·210 ±0·009	..	..
	Branch	34	2·560	..	..
	Flowering shoot	22	2·410	..	.
(d) Greentuft Islands (Fisherty How Bay)	Main axis	112	3·200 ±0·003	0·356 ±0·002	0·127
	Flowering shoot	38	2·070	..	..
(e) Congo Bay	Main axis	12?	3·200 ±0·050	0·553 +0·025	0·306
(f) Pullwyke Bay (Deep, 3–4 metres)	Main axis	56	2·700	.	..
	Branch	8	3·500	..	..
(g) Pullwyke Bay (Shallow, 0·5–1 metre)	Main axis	205	2·590 ±0·020	0·285 ±0·014	0·081
	Branch	17	1·660	..	..
	Flowering shoot	14	1·820	..	..

An examination of the data solely from the point of view oı the types of leaf-form shows that the leaves on the flowering branches are normally and relatively broader than those on the main axis. The other branches bear leaves which are generally of a similar L/B ratio to the main axis, though not necessarily so (*cf.* Pullwyke and Sawpit Bay samples). The production of branches in *P. perfoliatus* sometimes precedes the actual appearance of flower buds (*e.g.*, Low Wray samples) and may sometimes be more nearly associated with it in time [*e.g.*, Pullwyke (shallow) and Sawpit Bay samples]. In the former case it may be that the leaf shape resembles that of the main axis, while in the latter case it might be expected that the internal conditions would produce similar leaves on all developing branches whether flowering or not. This suggestion, however, requires further detailed examination.

Another noteworthy fact is that the maximum range of shape variation is shown by Coniston and Low Wray forms which possess the narrowest leaves. The range of shape variation on the other hand in case of broad leaved forms, *e.g.*, Wharfe and Pullwyke Bay (shallow) forms is comparatively narrow. This plasticity of the narrow leaves may be on account of a prolonged meristematic activity during which changes in growth conditions might be affecting leaf shape.

SHAPE VARIATION IN DEVELOPING LEAVES

A large number of young vegetative and floral buds was collected from stations at which variations in adult leaves were already studied. These buds were dissected and the young leaves measured by means of a micrometer under a microscope. The length of the developing leaves has been plotted against the breadth logarithmically for typical plant forms in Figs. 1 and 2. The curves apparently conform to the equation $x = cy^k$ as given by Pearsall (1927), where x and y are sizes of growing plant organs (corresponding to length and breadth in this study), c is a constant expressing their relative initial sizes and k is a quantity for their relative logarithmic growth rates. The value for k can be easily estimated from the slope of the curves so plotted.

Coniston forms as indicated in Fig. 1 show an initial high rate of relative growth for length with a k of the order of ± 6. However, the value for k falls down to $\pm 1 \cdot 1$ after the leaves have grown longer than 3 mm. The leaves mature so into the narrow forms. On the other hand the Wharfe type has a lower value of k (± 2) in the early phase of growth and hence develops into the broader form.

Measurements from buds taken from the top of about 6 inches high seedlings growing in Congo Bay are plotted in Fig. 2. Here the longest leaves are those first formed by the seedling ($k = \pm 1 \cdot 8$). The latter leaves show great variation but an average k of the order of ± 1. This may be held to indicate that as products of carbon assimilation become abundant k falls and this assumption would agree with the lower k for flowering plants as plotted on the same figure and as is shown subsequently for plants growing from rhizomes.
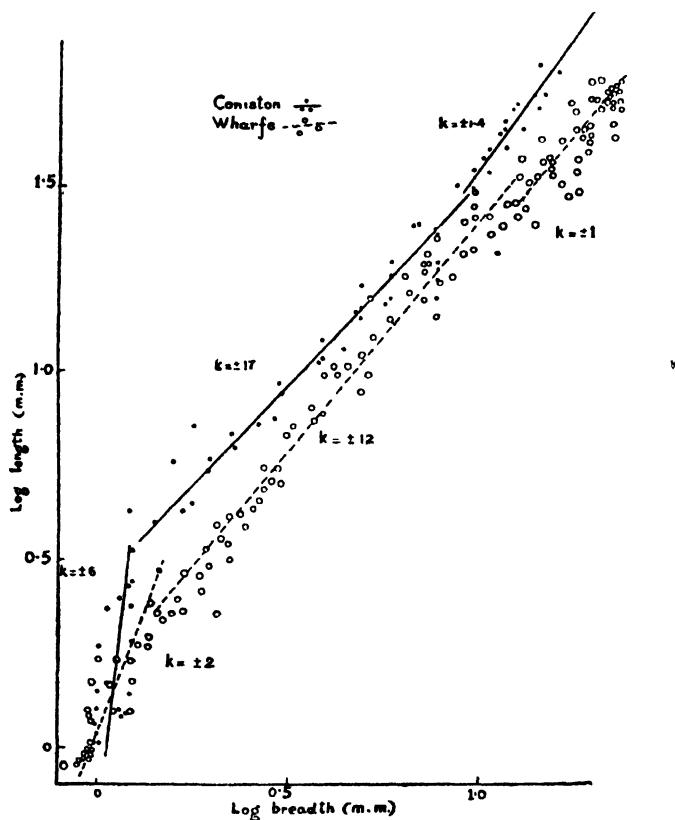
Fig. 1

Both of these in general possess broader leaves than the leaves upon a seedling or a non-flowering plant.

Two young plants apparently of the same age were found growing together from rhizome pieces of different thickness. The pieces were actually branches of the same parent rhizome. So the environmental conditions were identical for both of them. Nevertheless, the plant from the thicker rhizome had a thicker axis and the open leaves were also broader than those growing from the thinner rhizome. But on plotting logarithmic curves for the length and breadth of the young leaves from the two plants the same value for k ($\pm 1 \cdot 2$) was found for both the cases. Therefore differences in the shape of the leaves of the two plants must be on account of different values for c (in the formula $x = cy^k$) which would possibly depend upon different amount or kind of food supply from the rhizome during the stage when the leaf primordium was formed. If the food supply from the thicker rhizome is quantitatively superior in some respect to that from the narrow
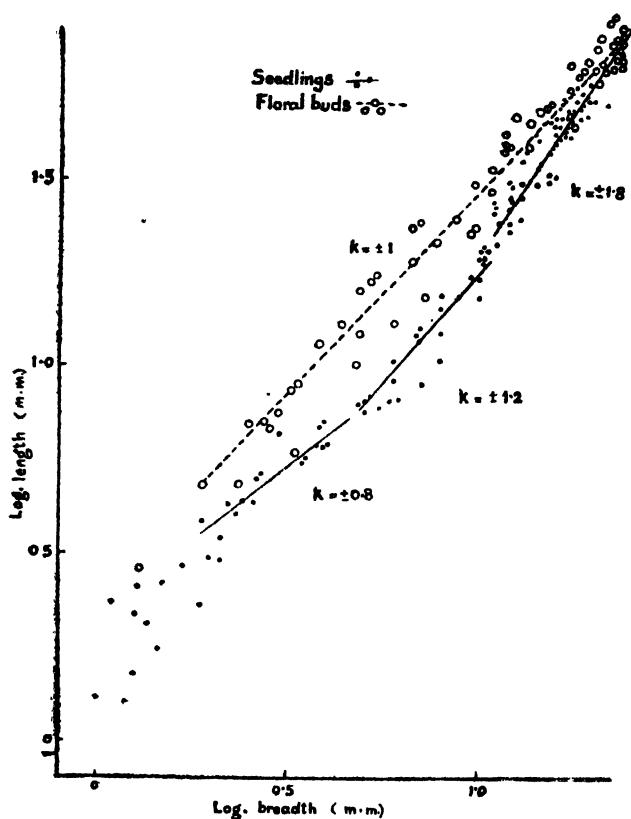
Fig. 2

one then the leaf-forms might be expected to be related to one another in much the same way as the successive leaves from a single rhizome. These become longer as they develop further from the rhizome. This is the difference observed in the examples between leaves from a narrow rhizome and those from a thick one.

As far as possible similar pieces of rhizome were obtained from Congo Bay and left in jars containing well water in the laboratory for a fortnight. The well water had a carbonate hardness two to three times more than the lake water. But when the buds developed from these rhizomes were dissected and the measurements were plotted against those developed at the same time in the lake no difference in the value of k or the shape of the leaves could be discovered. Hence it is clear that changes in the environment for a short duration cannot induce changes in the shape of the leaves which are just developing from the rhizome.

CHEMICAL ANALYSES OF PLANTS

In order to detect any obvious differences in chemical composition of the main food reserves air dried and pressed specimens were analysed for total nitrogen. The results are given in Table II. It is possible that soluble nitrogen may have been lost while pressing the plants between paper sheets and, during drying, some of the insoluble nitrogen might have been hydrolysed and redistributed in the plant. But such a preliminary study for the sake of comparison clearly showed that (*a*) buds of flowering plants have a lower nitrogen content than those from vegetative plants, (*b*) narrow leaves possess more nitrogen than broad leaves, and (*c*) the nitrogen content does not usually decrease much in older leaves of mainly vegetative plants.

TABLE II

Total nitrogen contents of air-dried and pressed specimens

(Expressed as percentage of dry weight)

	No. of estimations	Average	Standard deviation
(*a*) Buds—			
Vegetative	11	5·510 ±0·170	0·556 ±0·120
Flowering	7	4·550 ±0·110	0·428 ±0·160
(*b*) Narrow leaves (Coniston, Low Wray and Sawpit ; L/B = 3·2 -3·76)—			
Top leaves	12	4·510 ±0·101	0·350 ±0·073
Middle leaves	5	4·330 ±0·070	0·155 ±0·049
Bottom leaves	3	3·970 ±0·120	0·203 ±0·084
(*c*) Broad leaves (Pullwyke, shallow and Ullswater : L/B - 2·59 -2·92) —			
Top leaves	7	3·810 ±0·190	0·500 ±0·130
Middle leaves	1	3·900	.
Bottom leaves	1	3·600	.

For more accurate determinations freshly collected plants of comparable age were hung on a string in a room and when sufficiently dry in air they were transferred to an oven kept at a temperature of 65° C., where these were finally dried to a constant weight. The nitrogen content of these along with their L/B ratios are shown in Table III. It will be seen here also that narrow-leaved forms are generally richer in nitrogen than the broad leaved forms.

Further analyses were made in order to obtain a comparison between the food supplies of the growing regions. In this case buds were collected and dropped into boiling alcohol and boiled for ten

TABLE III

Total nitrogen content of whole plants

(Expressed as percentage of dry weight)

Locality	L/B of leaves from axis	Total nitrogen
Coniston	3·776 ±0·068	7·567
Low Wray Bay	3·654 ±0·019	6·253
Ullswater	2·920	5·318
Pullwyke	2·590 ±0·020	4·883

minutes, then cooled and stored. Subsequently the extract and the insoluble residue were analysed separately. The results are given in Table IV. The data clearly show that vegetative buds and their leaves have a higher proportion of soluble and insoluble nitrogen than developing leaves obtained from floral buds and branches and that the ratio total sugars/soluble nitrogen is higher for young leaves

TABLE IV

Chemical analysis of buds preserved in alcohol

(Results expressed as percentage of dry weight)

Form (Locality)	Material	Dry weight, % of fresh weight	Soluble nitrogen	Insoluble nitrogen	Total sugars	Reducing sugars	Total sugars / Soluble nitrogen
Coniston ..	Vegetative buds	3·083	1·897	8·690	17·490	7·703	9·217
Ullswater ..	Young leaves from flower buds	7·082	0·805	3·368	7·674	5·835	9·528
Fisherty How Bay	Young leaves from branches	3·699	2·317	8·245	11·330	4·196	4·890
	Flower buds	3·623	0·778	5·525	7·047	4·919	8·851
	Branch buds	5·269	0·909	6·243	8·307	5·388	9·139
Congo Bay ..	Young leaves from seedlings	6·348	0·399	3·417	10·940	4·169	27·370
	Young leaves from vegeta-tive buds	2·774	2·513	7·050	15·970	10·400	6·356
	Flower buds	6·078	0·250	2·802	3·968	2·917	15·890

obtained from floral buds or seedlings than for those obtained from vegetative buds. Thus both floral buds and seedlings seem to possess a high C/N ratio and both of them ultimately develop broad leaves. It also agrees with the general assumption that flowering is associated with higher C/N ratios.

DISCUSSION

It has been shown that shape variations in the leaves of *Potamogeton perfoliatus* are induced when they are still developing in the bud. The most potent factor likely to bring about such changes in the bud seemed to be the quantity and quality of food supply.

It is a well-known fact that the relative rates of growing plant organs can be altered by changes in the carbon to nitrogen ratio in the growing medium. For instance, Turner (1922) and Grist and Stout (1929) have obtained a high ratio of stem/root growth by supplying a high proportion of nitrogen to the plant. The behaviour of growth in length and that in breadth of the leaves can be taken to be of similar nature since they conform to the general equation $x = cy^k$ as formulated by Pearsall (1927) and discussed at great length by Huxley (1932). In the case of *P. perfoliatus* it is found that narrower leaves are developed in localities where the substratum contains well decomposed organic matter and as has been shown by Misra (1938) it contains more of nitrogen in the available form. Thus a high supply of nitrogen from the mud and its uptake by the plant seem to increase the relative rate of growth in the length of the leaves.

The most conclusive evidence of variation in the relative rate of growth by changes in the ratio of carbon/nitrogen supply comes from an analysis of the plants. This largely happens due to changes in the metabolic balance of the growing plant organs. A high C/N ratio for instance tends in dicotyledonous plants to turn meristem into vacuolating cells. This has been shown to some extent by Pearsall and Billimoria (1938) in case of sunflower. Pearsall (unpublished) has observed in case of Sycamore that a high C/N supply to vegetative buds gives rise to narrow, small and deeply lobed leaves and longer internodes. This phenomenon is attributed to early cessation of meristematic cell divisions and increased growth of the rippen meristem. But in monocots like *P. perfoliatus* where there is a basal meristem in the leaves for quite a long period it is difficult to see how increased post-meristematic growth can alter the shape of the leaf. Nevertheless, it seems very clearly from the data presented in this work that a high C/N supply tends to produce broad leaves in *P. perfoliatus* and not narrow ones as in the case of Sycamore.

The existence of a high C/N ratio in case of *P. perfoliatus* buds is possible in three different ways. Firstly low availability of nitrogen from the mud, secondly lower reserve of nitrogen in the young leaves when the plant is flowering as has been shown to exist and thirdly by a rapid translocation of carbohydrates from the rhizome to the developing young plant from it. In all these cases the leaf tends to develop broader.

Pearsall and Hanby (1925) have shown experimentally that the leaves of *P. perfoliatus* can be made to grow broader by an increased supply of calcium in their culture medium. Hence the rooting medium and the plant ash were also analysed in this work but the results are not recorded here since no significant correlation between these data and leaf shape could be established; yet there was some indication that replaceable calcium and iron in the mud favour growth in the breadth of the leaves. Soil conditions, if they have any effect upon leaf shape which must be very complex indeed, might control growth correlations through their effect upon carbon and nitrogen metabolism of the plant.

SUMMARY

Morphological, developmental and chemical studies of plants collected from different lakes and localities indicate that form-variation in *P. perfoliatus* is caused by an early differential growth ratio which is affected by the supply of carbohydrates to the growing organs. It has been shown that a high C/N ratio tends to decrease the differential growth ratio between length and breadth thus producing a broad leaf.

ACKNOWLEDGMENT

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LIFE-HISTORY AND MORPHOLOGY OF
TROCHODIUM AJREKARI GHARSE SP. NOV.

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INTRODUCTION

THE genus *Trochodium* was founded by Sydow (1919) to accommodate the rust occurring on *Ipomœa argyreoides* Chois, in the Cape Province of South Africa and, until quite recently, contained only one species— *Trochodium Ipomœœ* (Thuem) Syd. The urediospores are stated as not formed in the description given by Dietel (1928), which is evidently an error. Doidge (1926) found a few urediospores among the telio-spores in the more recent collection of the rust and it is manifest that they were present in the original specimen collected by MacOwan for Thumen who named the rust called the æcial stage *Aecidium Ipomœœ* Thumen and the uredial stage *Uredo aterrinia* Thumen, both names now included in the synonymy of the rust. That Thumen would not have given the name *Uredo aterrinia* without seeing the uredial stage goes without saying.

It may be added that Berkeley, apud Kalchbrenner (1882), found the telial stage of the rust in the same specimen which Thumen named *Uredo aterrinia* and presuming that the æcial, uredial and telial stages belonged to the same rust, he renamed it *Uromyces Ipomœœ* Berkeley, which name has been adopted by Doidge (1926), in preference to *Trochodium Ipomœœ*.

In a second species of the genus recently proposed by Thirumala-char (1942), *viz.*, *Trochodium Sampathense* on *Lettsomia elliptica* Wight and *Argyreia cymosa* Sw., pycnia, æcia and telia have been recorded ; but the rust appears to be an *opsis* form, for uredia have not been dis-covered and do not, presumably, occur.

A Eu-autœcious form of *Trochodium* on another member of *Convolvulaceœ*, to which family the genus seems to be restricted, is described in this paper. As far as the writer can see, the form described below is a new species which may be called *Trochodium Ajrekari* Gharse sp. nov. (?), pending its final identification. An amended description of the genus is also proposed, in view of the further discoveries of the different stages made since Sydow originally proposed it in 1919 and Dietel gave a diagnosis of it in 1928.

The writer found this *Trochodium* in October 1938 on a solitary plant of *Rivea hypocrateriformis* Choisy, on the slopes of Vetal Hill, near Poona. Though the hill abounded in plants of *Rivea hypocrateri-formis*, the rust which was found in the telial stage occurred only on

a single plant. The teliospores, it was noted, germinated immediately, without a period of rest, affording a clue that the rust was probably an autœcious form.

LIFE-HISTORY

This clue was followed up by inoculation experiments on *Rivea* seedlings. Young seedlings of *Rivea* raised in pots were inoculated with germinating teliospores. On the sixth day after inoculation yellowish white specks, smaller than the head of a pin, were seen on the upper surface. These specks were found only on young tender leaves. The older leaves did not show any sign of infection. Brownish yellow pycnial dots appeared on the 8th day, singly or in groups of 2–3, in the centre of the yellow spots previously noted. Later, the number of pycnia increased, forming round pustules. The pycnia were seen on both the surfaces. Aecial swellings were first observed on the lower surface on the 6th day after the appearance of the pycnia. In some cases the æcial swellings appeared eight days after the appearance of the pycnia. In all cases, the æcia completely ruptured on the 10th day after the appearance of pycnia, or 18th day after inoculation. A very few æcia appeared on the upper surface at a very late period, when most of the lower æcia were fully ruptured.

Fresh æciospores germinated within three hours. The germinating power of these spores declined gradually till, after a fortnight, very few spores germinated.

A set of *Rivea* seedlings was inoculated on the leaves with germinating æciospores with the following result :—

Yellow spots of about ½ to 1 mm. diam. were first observed on the 9th day after the inoculation and on the 10th day small, brown, irregular eruptions could be seen, under a hand lens, which later increased in number and size and were invariably arranged in concentric circles, usually two, surrounded by a yellow halo. These were the uredia. Urediospores also germinated at once within 5–6 hours and the germination decreased as time elapsed.

Germinating urediospores were inoculated on a fresh set of *Rivea* plants in the first week of September 1940. This also produced uredia ; but these, later, were found to be intermixed with teliospores. The percentage of teliospores increased as the time passed on.

These experiments with the *Rivea* rust indicate that it is an autœcious rust and an Eu-form, a fact confirmed later, by the discovery of a few æcia in nature on the solitary plant from which the telia were originally collected.

MORPHOLOGY

The *Trochodium* on *Rivea* showed all the spore forms on the hosts in the culture experiments. Opportunity is taken, accordingly, to give an amended description of the genus. The rust on *Rivea* is proposed as a new species, *Trochodium Ajrekari* (the specific name being intended to honour my teacher, Prof. S. L. Ajrekar). It shows some important differences from the forms previously described.

Trochodium Sydow, *Ann. Mycol. Berl.*, 1919, **17**, 106

Pycnia amphigenous, subepidermal, at first in small groups, later aggregated, erumpent, flask-shaped with ostiolar paraphyses. Aecia amphigenous, but usually epiphyllous, in circles around the central group of pycnia, cupulate, with well-developed peridium ; aeciospores in loose chains, polyhedral to rounded, ranging in diam. from 20 to 36 µ with one germ-pore. Uredia amphigenous, in circular patches, without paraphyses : urediospores hyaline, echinulate, ranging in diam. from 28-38 µ, with equatorial germ-pore. Telia amphigenous, subepidermal, erumpent, scattered, minute, without paraphyses : teliospores one-celled, almost black, flattened globose, ranging in breadth from 26–33 µ and height 24–28 µ, with a flattened, fuscous papilla: epispore with regular longitudinal striæ, radiating from apex ; germ-pore apical ; pedicel hyaline, persistent, swelling in water ; germinating without a rest-period into a four-celled promycelium.

Type species.—Trochodium Ipomœæ (Thumen) Sydow, on *Ipomœæ argyreoides* Choisy, in Cape Province, South Africa

Trochodium Ajrekari Gharse, Sp. Nov.

Pycnia amphigena, primo 1 3, deinde plura simul in catervas circulares coacta ; singulæ pycnium ca. 1-1·5 mm. diam., amphoræ similes, primo obscure flavæ, deinde brunneæ ; pycnia 100–140 µ diam. Ostiolum eminens, paraphysibus ornatum. Aecia epiphylla, subepidermalia, erumpentia, circulariter coacta circa, pycnia aggregata in centro, cupulata, tenuiter flava, 200–300 µ, peridio prominenti. Peridii cellulæ oblongæ vel polyhedrales, 26–33 × 19–30 µ, pariete externo dense tuberculato : æciosporæ angulariter, globosæ, tenuiter flavæ vel hyalinæ, verrucosæ, diam. ex 22 ad 36 µ, sæpissime 26–31 µ. Uredia amphigena, diam. 3–5 mm., circumdata absque exceptione corona flava ; urediosporæ aurei coloris, 28–38 µ diam., plerumque 30–34 µ, epispora hyalina, echinulata, germinationis poro in regione equatoriali absque paraphysibus. Telia amphigena, subepidermalia, erumpentia ; teliosporæ interdum mixtæ urediosporis, obscure brunneæ, complanate globosæ, amplioris latitudinis quam longitudinis, latitudinis 26–33 µ, plerumque 28–31 µ, longitudinis 24–26 µ, raro 28 µ, ornatæ striis quæ radiorum instar emergunt ex apice et convergunt ad basim ; germinationis porus apicalis, pediculus hyalinus, persistens, longitudinis 60–65 µ, superiori parte tumescente in aqua atque cystum aliqualem efformante ; cystus 19–37 µ diam., statim germinans in 4-cellulatum promycelium.

Habitat in follis vivis *Rivea hypocrateriformis* Choisy. Typum invenit Gharse in clivo Vetal, Poona, mense Octobri 1938, atque deposuit in Herb. Crypt. Ind. Orient., in Imp. Agric. Res. Instit., New Delhi.

Trochodium Ajrekari GHARSE, SP. NOV.

Pycnia amphigenous, at first in groups of 1–3, later forming rounded groups of larger numbers, each about 1-1·5 mm. in diameter ; flask-shaped, dark yellow at first, later brown, 100–140 µ in diameter ; ostiole prominent, with ostilar paraphyses. Aecia epiphyllous, subepidermal,

erumpent, arranged in circles round the central group of pycnia, cupulate, faint yellow, 200–300 μ, with a prominent peridium ; peridial cells oblong to polyhedral, 26–33 × 19–30 μ, outer wall densely tuberculate ; æciospores angular globose, yellowish to hyaline, verrucose, ranging in diameter from 22–36 μ, mostly 26–31 μ. Uredia amphigenous, measuring 3–5 mm. in diameter, invariably surrounded by a yellow halo ; urediospores golden yellow, 28–38 μ in diameter, mostly 30–34 μ,
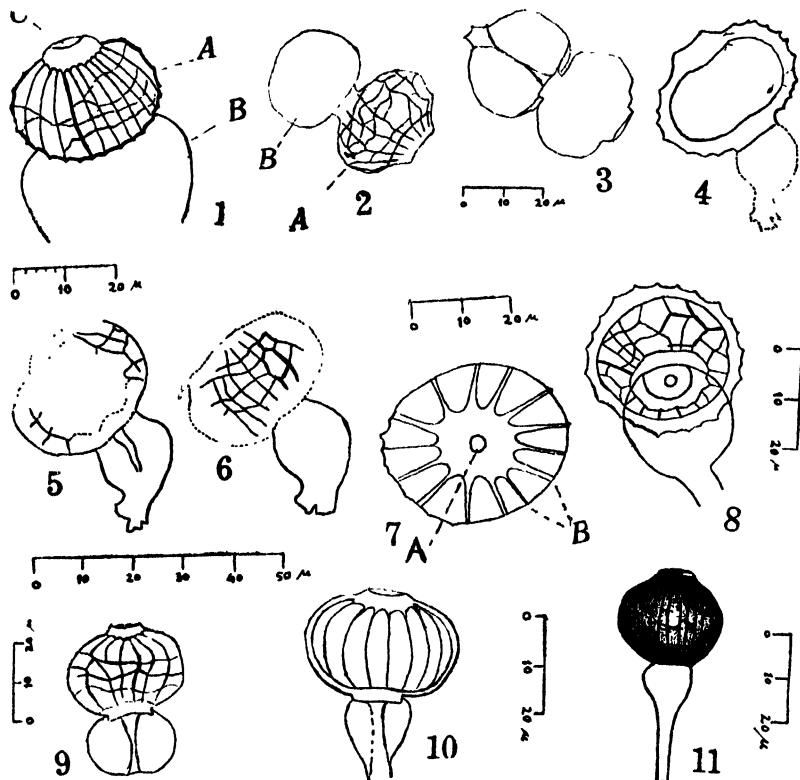
Figs. 1—11.—Every figure is accompanied by a scale of its magnification. The figures relate to *Trochodium* on *Rivea hypocrateriformis* unless otherwise specified.

Figs. 1 and 2. Teliospores in side view showing reticulate surface markings. (Drawn by combining different optical sectional views to bring out all the markings.) (A) Spore proper ; (B) Cyst ; (C) Germ pore. Fig. 3. The same spore as in 2 in outline (Side view). Figs. 4–6. A mature teliospore in three optical sections. Fig. 7. Top view of a teliospore showing the germ pore (A) and the longitudinal striæ (B). Fig. 8. Bottom view of a teliospore (slightly tilted) showing the attachment of the cyst and also the surface markings. Fig. 9. Markings on the surface of a mature teliospore of *Trochodium Sampathense*. (Several optical sections are combined here. A mature teliospore of *T. Ipomœæ* presents an exactly similar appearance.) Fig. 10. An immature teliospore of *T. Sampathense*. Only longitudinal markings are seen. Fig. 11. A teliospore of *T. Sampathense*. [As reproduced from Thiruma lachar (1942).]

with hyaline, echinulate epispore and a germ-pore at the equator and without paraphyses.   Telia amphigenous, subepidermal, erumpent ; teliospores sometimes intermixed with urediospores, dark brown, flattened globose, broader than long, ranging in breadth from 26–33 μ, mostly 28–31 μ, height 24–26 μ, rarely 28 μ, with regular striæ radiating from the apex and converging towards the base ; germ-pore apical ; pedicel hyaline, persistent, 60–65 μ long, upper part swelling in water forming a kind of cyst ; cyst 19–37 μ in diameter ; germinating at once into a 4-celled promycelium.

Hab. on living leaves of *Rivea hypocrateriformis* Choisy, Vetal hill, Poona, Oct. 1938, Gharse (type).   Type deposited in Herb. Crypt. Ind. Orient. of the Imperial Agricultural Research Institute, New Delhi.

From *Trochodium Ipomœæ*, the present species differs in having smaller æcia, but larger æciospores ; larger urediospores, and much larger telial pedicels.   The cysts of *T. Ipomœæ* are moreover much smaller.   *Trochodium Sampathense* differs from *Trochodium Ajrekari* in being an opsis form as proved in his culture experiments by Thirumalachar (1942) and in having smaller æciospores and teliospores.

The earlier descriptions of the markings make no reference to any lines (ridges) of latitude which in combination with longitudinal lines give a distinctly reticulate appearance to the markings with ridges enclosing alveoli or areolæ (Figs. 1, 2, 8 and 9).   On closer study of the African and the South Indian specimens, however, these lines (ridges) of latitude were noticed in both, particularly in darker mature spores.   In the writer's experience the colour of the mature teliospores is distinctly darker than that of immature ones and he suspects that the previous observers of *Trochodium Spp.* have failed to notice the latitudinal markings on the teliospore walls, probably because they examined only the younger spores (Fig. 10).   Even in mature spores the latitudinal ridge could not be seen clearly when longitudinal ridges were focussed.   Thirumalachar's (1942) fig. 6 shows some transverse ridges but the pattern is rather different from that observed by the writer (compare Fig. 1 with fig. 11 reproduced from Thirumalachar).

SUMMARY

A species of *Trochodium* was discovered on *Rivea hypocrateriformis*, occurring on the Vetal hill near Poona.   In cultural studies it was found to be an antœcious rust, showing all stages—pycnia, æcia, uredia and telia—in succession.   An amended description of the genus to include all these stages is, therefore, given.

The inflated cyst at the base of the teliospore and the character-istic markings on the spore itself easily led to the identification of the form as a species of the genus *Trochodium*.   A careful comparison of the African specimen—*Trochodium Ipomœæ* (Thuem) Syd., of *T. Sampathense* Thirumalachar and of *Trochodium* on *Rivea* was made.   No pycnia are recorded for the African species and no uredia have been observed in connection with *T. Sampathense*.   The spore-measurements

of the three forms also show sufficient differences leading to the con-
clusion that the *Trochodium* on *Rivea* is a new species for which the name
Trochodium Ajrekari is proposed.

In conclusion, the writer wishes to express his indebtedness to
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AN *ALTERNARIA* DISEASE OF SAFFLOWER*

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INTRODUCTION

SAFFLOWER (*Carthamus tinctorius* L.), an annual herbaceous plant with large orange-coloured flower heads, is grown in many parts of the world, Southern Europe, Egypt, Persia, India, China, Southern Rhodesia and South America. In India it is cultivated in Northern, Eastern, Central and Western India for its florets which are the source of a reddish dye, *carthamin*, and for its seeds from which an oil of considerable commercial importance is extracted. Safflower seed cake, a by-product during the extraction of oil, is used as a fertilizer.

In 1936 a leaf-spot disease due to a species of *Alternaria* was noticed in this crop in the Botanical Section of the Imperial Agricultural Research Institute at Pusa. It was common in the cultivators' fields at Pusa, Samastipur, Dharbhanga, Patna and Muzaffarpur wherefrom specimens were obtained and examined. It has been reported from the Central Provinces[1] but is evidently unknown in any other part of the country.

The extent of damage caused by it appears, however, to be very slight.

SYMPTOMS OF THE DISEASE

The disease first makes its appearance just before flowering and is manifest on all parts of the plant especially the leaves.

In the beginning minute brown to dark brown spots, one to two millimetres in diameter, with concentric rings appear on the leaves. The diameter of the spots gradually increases to about one centimetre. Very often two or more adjacent spots coalesce and form large irregular lesions. The spots gradually become darker on account of the formation of the fructification. The central portion of the spot is generally light brown and is surrounded by a number of dark rings alternating with light ones. With the maturing of the spots, shot holes appear in the infected areas and if the whole leaf is attacked the blade breaks in an irregular manner due to the brittleness of the dead tissue.

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* Major portion of the work was carried out by the author in the Mycology Section of the Imperial Agricultural Research Institute.

[1] Private communication from the Mycologist, Central Provinces.

The disease is less severe on the stem and petiole where the spots are elongated. If the parasite attacks the flower buds they fail to open. Minute dark brown spots first appear at the base of the calyx ; these spots enlarge, spread and later attack other parts of the flower. The unopened flowers shrivel and dry up. Fig. 2 shows the symptoms of the disease.

MORPHOLOGY OF THE PARASITE ON THE HOST

Mycelium.—The mycelium of the organism in the tissues of the infected area is septate and inter- and intra-cellular, with slight constrictions at the septa. The hyphæ, when young, are sub-hyaline, narrow and sparsely septate but when mature they are dark coloured, more frequently septate and broader.

Conidiophore.—The conidiophores are formed on the central dead portion of the spots. They are stout, erect, rigid, unbranched, septate and slightly constricted at the septa. They arise singly or in clusters bursting the epidermis or through the stomata, and are brown to olivaceous in colour (except the tip which is almost hyaline), about 6 to $10\,\mu$ in width, septate (the number of septa varying from 0 to 5) and rounded at the tip which is marked by a single terminal scar ; a lateral scar is sometimes also visible. The length ranges from 15 to $85\,\mu$. Sometimes a spherical swelling (upto $12\,\mu$ in diameter) is seen at the base of the conidiophore.

Conidia.—The conidia (Fig. 1) are irregular in shape, with an apex, which is usually blunt, though tapering. Some of the smaller spores are roughly spherical and others elongate-cylindrical with rounded ends. The basal scar is usually plainly visible as also a definite
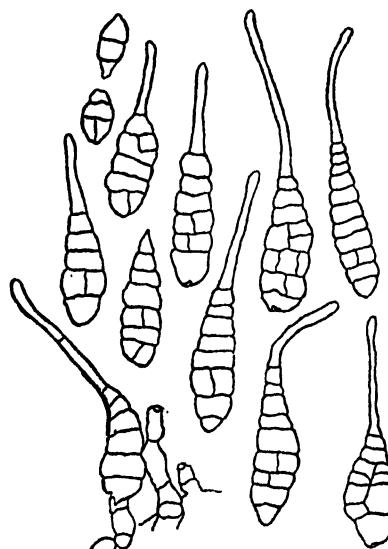

Fig. 1. Conidia of *Alternaria carthami* from nature (× 520).

apical scar, showing that a chain of at least two spores must have occurred. The conidia are light brown, translucent and a majority of them possess a long beak. They measure 36 to 171 μ with the beak, and 36 to 99μ without the beak, in length, and 12 to 28 μ in width. The conidia are usually 3 to 11-celled ; longitudinal septa are common, their number has been found to vary from 0 to 6. The beak is very lightly brown near the base and almost hyaline at the apex ; it measures 15 to 84 μ in length and 3 to 5 μ in width. A few spores without any beak sometimes occur. The surface of the conidia is smooth but sometimes with a granular appearance is surface view.

ISOLATION AND INFECTION EXPERIMENTS

The infected parts always showed the presence of olivaceous brown mycelium and spores of *Alternaria* species. Several single spore isolations were made and all isolations were found to be identical.

(i) *Inoculation on the Host.*—Inoculation experiments were performed on plants which had been grown from sterilized seeds. Two different methods of inoculation were followed : plants were either sprayed with spore suspension in sterile water or after spraying the plants with sterile water masses of culture containing spores and mycelium were placed on the required spots with a sterilized needle. The plants were kept covered by bell-jars for 24 or 48 hours and kept moist by occasional spraying with sterile water.

In another series, plants were inoculated by both the methods described above but they were not covered with bell-jars. The results of the inoculation experiments are summarised in Table I.

TABLE I

Summary of the results of inoculation experiments on Safflower by Alternaria *sp.*

Method of inoculation	Whether covered or not by bell-jars	No. of plants inoculated	No. of plants infected	No. of control plants	Controls infected
Mycelium and spores ..	Covered	72	70	32	Nil
Do. ..	,,	60	60	28	,,
Do. ..	Not covered	42	14	19	,,
Do. ..	,,	27	9	12	,,
Spore suspension ..	Covered	67	55	32	,,
Do. ..	,,	74	70	31	,,
Do. ..	Not covered	28	7	10	,,
Do. .	,,	37	12	17	,,

It will be observed from the data presented in Table I that the presence of moisture is essential for infection ; very few plants took infection when they were not covered by bell-jars even though they were sprayed with sterile water from time to time. The method of inoculation made no difference since infection readily occurred whether

the inoculum was applied as a spore suspension or as a mass of mycelium and spores. The fungus was in every case reisolated from the infected plants. Controls were kept but in no case did they become infected.

(ii) *Cross Inoculations.*—Cross inoculation experiments were carried out on hosts which are common hosts of *Alternaria* sp. The results are recorded in Table II.

TABLE II

Cross inoculation experiments with safflower Alternaria

Hosts	No of plants inoculated	No of plants took infection
Potato (*Solanum tuberosum* L.)	27	Nil
Tomato (*Lycopersicum esculentum* Mill.)	17	,,
Cucumber (*Cucumis staivus* L.)	15	,,
Cotton (*Gossypium herbaceum* L.)	29	,,

Data presented in Table II show that the species of *Alternaria* isolated from safflower does not infect potato, tomato, cucumber and cotton plants.

GROWTH IN CULTURE

The fungus was grown on oat meal agar, Hopkin's agar, Dox's agar, potato-dextrose agar, Brown's standard synthetic agar and malt extract agar at room temperature (28–30° C.). The linear rate of growth was practically the same in the first four media : it was slightly less in malt-extract agar and the least in Brown's standard synthetic agar.

In all the media in which the fungus was cultivated there was very little aerial mycelium. Mycelial growth was mostly submerged. Sometimes a tuft of floccose dark green aerial mycelium was seen around and about the inoculum. The submerged mycelium also gave the media a dark green colour. The colour around the colony was mostly white except in the potato-dextrose agar where it showed a slightly yellowish tint.

TEMPERATURE RELATIONSHIP

The linear rate of growth of the safflower *Alternaria* was studied on Hopkin's agar at various temperatures. The experiment was carried out in selected petri-dishes of uniform size into which equal amounts of the medium were poured. All the dishes were inoculated at the same time and kept at various temperatures in darkness. The experiment was run in triplicate and repeated twice. The diameters of the colonies after seven days growth are presented in Table III.

TABLE III

Growth of safflower Alternaria *at various temperatures*

Temperature (° C.)	Diameter of the colonies (mm.)
15	24
20	26
25	42
30	45
35	6

It will be observed from the data presented in Table III that the optimum temperature for growth lies between 25° and 30° C.

HYDROGEN-ION CONCENTRATION

Richards' solution as modified by Karrer and Webb (1920) was used for studying the growth rate of the fungus at different hydrogen-ion concentrations. 30 c.c. of the solution together with the required amount of N/5 acid and N/5 alkali and distilled water to make 50 c.c. was put in each flask and hydrogen-ion concentration was determined according to the colorimetric method of Clark and Lubs (1917). Four flasks were prepared for each pH value. The flasks were inoculated with a young growing culture of the fungus and incubated at room temperature (28—30° C.). After 30 days the dry weight of the mycelium was determined and is shown in Table IV.

TABLE IV

Growth of safflower Alternaria *in Richards' solution at different hydrogen-ion concentrations*

Hydrogen-ion concentration	Dry weight (mg.)
3	121
4	176
5	207
6	478
7	369
8	292
9·2	145

From the data presented in Table IV it will appear that the fungus can grow in a wide range of hydrogen-ion concentrations. The range of optimum reaction appears to lie between pH 6 and 7 and the amount of mycelium produced is the greatest at pH 6.

IDENTIFICATION OF THE PARASITE

Hitherto no species of *Alternaria* has been reported on *Carthamus tinctorius*. From a consideration of the morphology of the spore and the beak the nearest species to safflower *Alternaria* have been found

to be *Alternaria solani* (Ell. & Mart.) Jones & Grout, *A. tomato* (Cooke) Weber, *A. cucumerina* (E. & E.) Elliot and *A. macrospora* Zimm. But from the reported spore measurement data presented in Table V it will become clear that the *Alternaria* under study does not agree with any one of them.

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TABLE V

Comparison of spore measurement data of safflower Alternaria
with other related species of the genus

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Fungus	Length (μ)		Total length including beak	Width (μ)
	Conidial body	Beak		
A. solani (Ell. & Mart.) Jones & Grout.	..	..	120–296	12–20
A. tomato (Cooke) Weber	..	..	100– 20	20–22
A. cucumerina (E. & E.) Elliot	30–75	25–35	55–110	15–25
A. macrospora Zimm.	..	..	150– 70	20
Safflower *Alternaria*	36–99	12–84	36–171	12–28

Some diseased leaves of safflower and a culture of the *Alternaria* sp. were sent to Dr. S. P. Wiltshire, Director, Imperial Mycological Institute, Kew, England. According to him the fungus came nearest to *A. macrospora, A. tomato, A. solani* and *A. cucumerina* but he concluded that the safflower fungus does not exactly match with any ·one of them.

Cross inoculation experiments carried out have shown that the *Alternaria* on safflower does not attack potato, tomato, cotton and cucumber plants. This together with the spore measurements seems to justify the establishing of this fungus as a new species for which the following name is suggested :

<div align="center">

Alternaria carthami CHOWDHURY SP. NOV.

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Alternaria carthami sp. nov.—Vegetative hyphæ septate, inter- and intra-cellular, when young sub-hyaline, narrow, sparsely septate, but when mature dark coloured, more frequently septate, broader. Conidiophores stout, erect, rigid, unbranched, septate, slightly constricted at the septa, arising singly or in clusters bursting the epidermis or through the stomata, brown to olivaceous in colour, 6 to 10 μ in width. Conidia light brown and translucent, muriform, formed at the tips of the conidiophores singly or in chains, 3 to 11-celled, longitudinal septa few, usually possessing a long beak ; conidia measure 36 to 99 μ ×12 to 28 μ without the beak. Beak very lightly brown near the base and almost hyaline at the apex, filiform, measuring 12 to 84 μ ×3 to 5 μ.

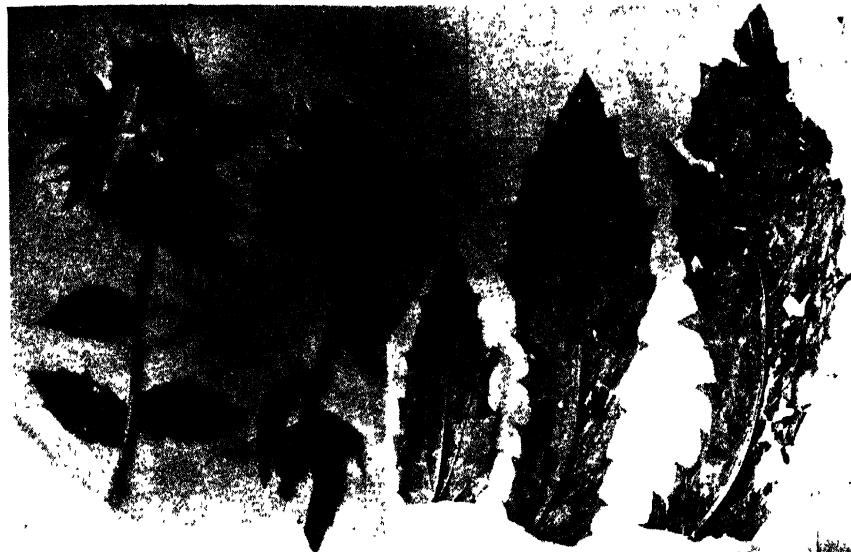*Habitat.*—Parasitic on the leaves and stems of *Carthamus tinctorius* at Pusa.

Fig 1. Flowering twigs and leaves of *Carthamus tinctorius* showing symptoms of the disease.

S. CHOWDHURY—

AN ALTERNARIA DISEASE OF SAFFLOWER

Type specimen collected by S. Chowdhury in January, 1936 and deposited in the Herbarium *Cryptogamæ Indiæ Orientalis*, Imperial Agricultural Research Institute, New Delhi.

Alternaria carthami Chowdhury sp. nov.—Hyphis vegetativis septatis, inter et intra-cellularibus, primo subhyalinis, angustis, sparse septatis, aetate fuscis et sæpius septatis, latioribus. Conidiophoris crassis erectis, rigidis, simplicibus, septatis, ad septum tenuiter constrictis, singularis vel fascicularis emergentibus, ex epidermide erumpentibus vel per stomata emergentibus, brunneis, 6–10 m diam. Conidiis pallidebrunneis, pellucidis, muriformibus, ad apicibus conidiophororum singularibus vel catenulatis formantibus, 3–11 cellularis, septis longitudinalis paucis plerumque rostris longis, conidiis 36–99 × 12–28 M rostris excluderentibus. Rostro pro parte basili pallidebrunneis, pro parte apic prope hyalino filiformi, 12–84 × 3–5 M.

Habitat in foliis *Carthamus tinctorius* L. Pusa.

Typus in Herbarium *Cryptogamæ Indiæ Orientalis*, Imperial Agricultural Institute, New Delhi, Leg. S. Chowdhury, January, 1936.

SUMMARY

A leaf-spot disease of *Carthamus tinctorius* was observed at Pusa and its neighbourhood. It has also been observed in other parts of Bihar and in the Central Provinces.

The disease was found to be caused by a species of *Alternaria* hitherto not reported from any part of the world. The morphology and parasitism of the fungus have been studied. It is proposed as a new species for which the name *Alternaria carthami* Chowdhury sp. nov. has been suggested.

The optimum temperature for growth has been found to lie between 25° and 30° C. and the range of optimum hydrogen-ion concentration for growth between 6 and 7.

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THE EMBRYO-SAC AND THE EMBRYO OF *SATYRIUM NEPALENSE* DON.

BY B. G. L. SWAMY

THE genus *Satyrium* Swartz, of the sub-tribe Diseæ belonging to the Tribe Ophrydeæ (Hooker, 1894), consists of about 50 species, which are mostly confined to Africa. *Satyrium nepalense* Don. is the only species reported from India. This plant forms a part of the characteristic vegetation of the grassy hill-top flora of the Deccan.

The plant has a subterranean unbranched tuber which commences its vegetative activity with the advent of the rainy season and the flowers are borne between July and September. The colour of the flowers varies from flake-white to deep mauve in different plants. The material for the present investigation was collected from Kodaikanal and Bababudan regions.

MEGASPOROGENESIS

The origin of the female archesporial initials in the ovary can be recognised while the pollinia of the same flower show the mature 2-nucleate condition which is the shedding stage. The archesporial cell differentiates sub-epidermally and becomes conspicuous with rich cell contents. This functions directly as the megaspore mother cell (Fig. 1).

While the megaspore mother cell is in the stages of the meiotic divisions, the organisation of the inner integument consisting of two layers of cells will be complete and the outer one will have made its appearance. Simultaneously with the completion of the 8-nucleate embryo-sac, the outer integument grows beyond the inner one leaving a space between them and completes its development (Fig. 7).

The megaspore mother cell by reduction divisions gives rise to a linear row of four megaspores (Fig. 2). Chromosome counts made on the metaphase plates of the megaspore mother cell showed 41 univalents (Fig. 13). Occasionally the micropylar dyad cell divides in an oblique plane. The second reduction division follows the first immediately and the dyad cells and the four megaspores are all equal in size as soon as they are formed ; and the enlargement of the chalazal megaspore commences subsequently. In this respect this plant differs from the rest of the investigated members of its Tribe, Ophrydeæ (genera like *Orchis, Habenaria, Herminium,* etc.), wherein at the dyad stage itself there is a differentiation in size of the two cells, the micropylar cell being very much smaller than the chalazal one ; even the subsequent development of the micropylar dyad cell is most variable, sometimes not dividing at all or sometimes exhibiting a

belated development and the division product of the lower dyad cell also in the above-mentioned genera shows distinct unequality in size.

The lowermost megaspore towards the chalaza resolves itself into the mature 8-nucleate embryo-sac following the normal type of development (Figs. 3 to 6). Occasionally the mature embryo-sac contains only 6 nuclei (Fig. 4) due to the failure of division of the antipodal nuclei at the 4-nucleate condition of the sac. Under such circumstances the antipodal nuclei remain very much diminished in size. In the normal 8-nucleate sacs the antipodal nuclei organise into definite cells (Fig. 6). Synergids are organised from the division of one nucleus and the egg and the upper polar nucleus from another nucleus of the micropylar group of the four-nucleate stage.

Double fertilisation (Fig. 8) takes place quite normally and the fusion of the second male nucleus with that of the unfused polars is complete but degeneration sets in soon after fusion. .
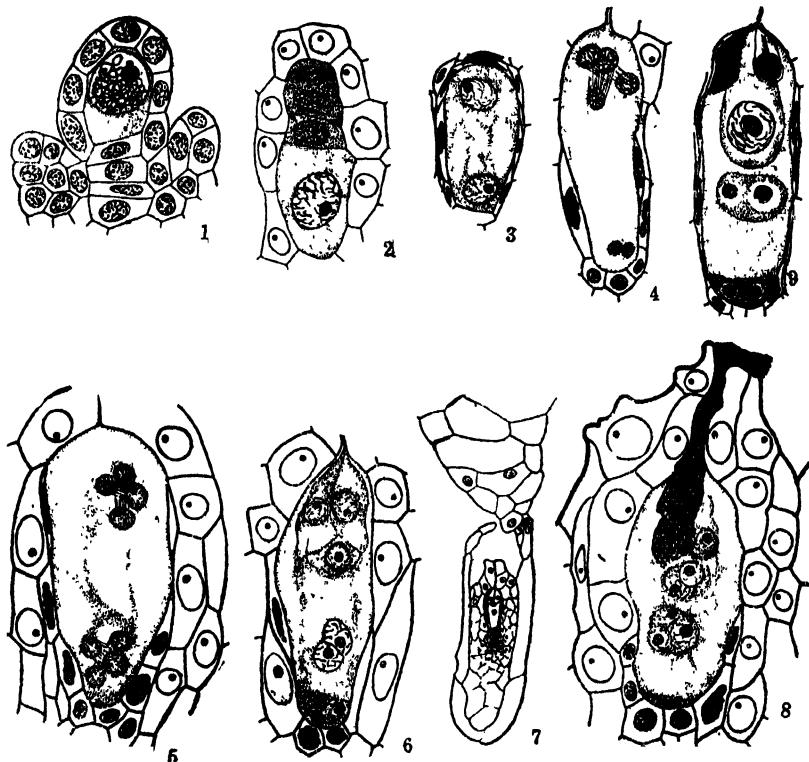
Figs. 1 to 9.—Fig. 1. Megaspore mother cell in synizesis (× 1260). Fig. 2. Linear tetrad or megaspores, the chalazal one enlarging (> 1800). Fig. 3. 2-nucleate embryo-sac (× 1260). Fig. 4. 6-nucleate embryo-sac; note the small size of the antipodal nuclei (× 1800). Fig. 5. 8-nucleate un-organised embryo-sac (× 1800). Fig. 6. Fully organised 8-nucleate embryo-sac (× 1800). Fig. 7. Longitudinal section of a seed when the embryo-sac is ready to be fertilized (× 400). Fig. 8. Double fertilization (× 1800). Fig. 9. Zygote (× 1800).

EMBRYO

The zygote (Fig. 9) divides by a transverse wall (Fig. 10). The subsequent development is highly variable. Sometimes the terminal cell again divides by a wall parallel to the first and thus a proembryo of a chain of three cells may be organised. The terminal cell of the proembryo divides first by a vertical wall and subsequently by walls in all planes and contributes to the organisation of the embryonal mass of cells. Usually the basal cell, sometimes also the middle cell and at other times both divide and contribute to the formation of a filamentous suspensor consisting of 4 to 5 cells (Figs. 14, 16 and 17). This organ occasionally elongates beyond the outer integument. In the majority of cases the suspensor does not extend out but becomes bent on itself and ultimately crushed between the embryonal mass and the limiting membrane of the embryo-sac (Fig. 18). In rare instances the terminal cell of the proembryo divides by a vertical wall and a suspensor as figured in Fig. 15 is organised. It is very common to find in the suspensors of the types mentioned above all stages of arrested development.

In certain ovules after the first transverse division of the zygote, the lower cell divides by vertical and later by oblique walls and forms the embryonal mass. In such cases the basal cell very slightly enlarges and remains very inconspicuous.

In still other ovules simultaneous divisions take place in both the cells of the two-celled proembryo in various planes to result in the mature embryo. Hence the organisation of a suspensor is completely suppressed. Some of the stages illustrating this type of development have been figured in Figs. 11 and 12.

Whatever may be the course of development of the embryo, all the mature embryos in the seeds develop to the same extent in size and differentiation ; whether it has at any time of its development no suspensor or a suspensor consisting of a single cell or of more than one cell is immaterial. It is very difficult to postulate any hypothesis for this uncertain behaviour of the suspensor organ, especially when one considers the fact that all the above-mentioned types occur in the ovules of one and the same ovary.

In this connection a comparative account of the structure of the embryo in the preceding sub-tribe Habenarieæ and the following Tribe Cypripedieæ of the present sub-tribe Diseæ (to which *Satyrium nepalense* belongs) may be considered. In all the investigated species of the genus *Habenaria* (Swamy, in press) of the preceding sub-tribe Habenarieæ, the zygote normally develops a proembryo of 3 cells as in many other orchids. The basal cell and the middle cell give rise to a filamentous suspensor of 5 to 7 cells in number. The cell of the suspensor towards the micropyle soon grows out of the outer integument, embeds itself in the placental tissue, develops lobations, and acts as an aggressive haustorium. The terminal cell of the proembryo by further divisions develop into the body of the embryo. According to Treub (1879), species of *Herminium* (the other preceding genus of the subtribe Habenarieæ) also show a very similar type of development.

In *Cypripedium* (Tribe Cypripedieæ) which is the genus next to *Satyrium*, according to Treub (1879) a rudimentary suspensor of 2 to 3 cells is developed in *Cypripedium venustum* and *C. barbatum.* In *C. parviflorum* (Carlson, 1940), the suspensor consists of a single cell " which eventually disappears ". Her figures are very vague and do not convey definitely the presence of a single-celled suspensor. In *C. reginæ* (Schnarf, 1931) a suspensor is characteristically absent.

It will be seen from the preceding account that the development of the embryo and suspensor in *Satyrium nepalense* exhibits characteristics of certain genera of its preceding subtribe Habenarieæ and the following genus *Cypripedium* of the next Tribe Cypripedieæ. Thus the plant under question may be considered as an intermediate form from the point of view of embryological evidences as revealed in the present investigation.
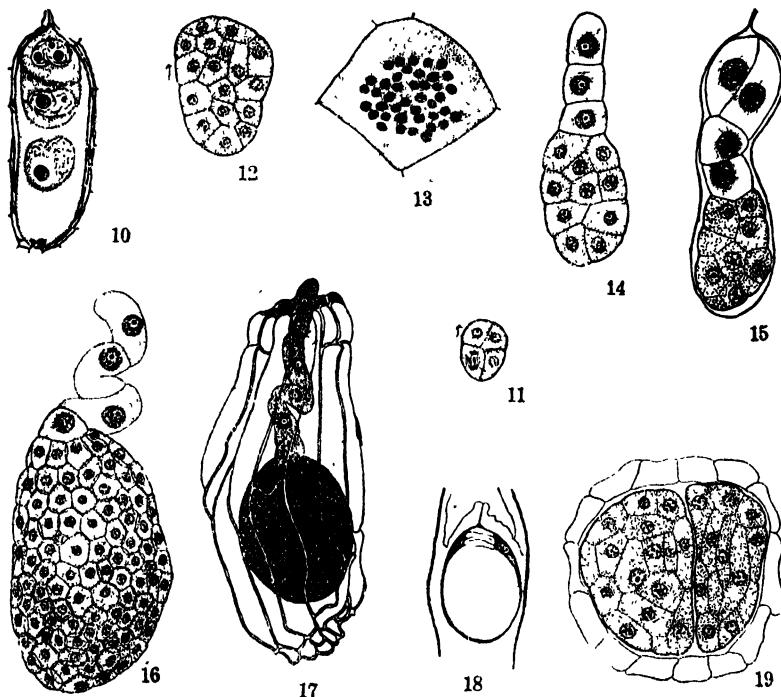
Figs. 10 to 19. —Fig. 10. 2-celled embryo (× 1800). Figs. 11 and 12. Stages in the development of suspensor-less embryos ; the arrows point toward the micropylar pole (× 1260). Fig. 13. Metaphase plate of the megaspore mother cell during the meiotic divisions (× 3600). Fig. 14. Young embryo with a suspensor made up of three cells (× 1260). Fig. 15. Young embryo with a " T-shaped " suspensor (× 1260). Fig. 16. A normally developed mature embryo (× 1260). Fig. 17. Semi-diagrammatic representation of a mature seed with its transparent seed-coat (outer integument) ; the embryo in this case shows its suspensor slightly protruding out of the outer integument (×400). Fig. 18. Diagrammatic section of an embryo showing the crushed suspensor between the embryo-sac membrane and the embryo (× 400). Fig. 19. Transverse section of a seed containing two embryos (× 400).

One instance was seen where a mature seed contained two embryos (Fig. 19) ; the exact relation of these could not be ascertained as this seed was cut transversely and this was the only one instance of polyembryony noticed.

SUMMARY

The embryo-sac develops according to the normal type, though 6-nucleate mature sacs are of occasional occurrence. The haploid chromosome number is 41. The development of the suspensor is very variable, exhibiting all stages in the reduction of the suspensor organ and finally completely eliminating it from some embryos. When embryological evidences are taken into consideration it is manifest that *Satyrium nepalense* may be looked upon as a form holding an intermediate position between the sub-tribe Habenarieæ and the Tribe Cypripedieæ.

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A CONTRIBUTION TO THE ANATOMY OF *SALVADORA PERSICA* L. WITH SPECIAL REFERENCE TO THE ORIGIN OF THE INCLUDED PHLOEM[1]

BY BALWANT SINGH

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1. INTRODUCTION

LITTLE is known about the exact mode of origin of the included phloem in angiosperms. In their review of plant anatomy, Eames and MacDaniels (1925, p. 258) state that there are two methods by which groups of phloem cells become embedded in the secondary xylem. In genera like *Combretum* and *Entada*, the cambium, besides cutting off secondary phloem on its outer side, also gives rise to isolated strands of phloem towards the inside in place of xylem cells which are normally produced. After a brief period of such activity these cambium segments return to their normal function, and thus bury the inwardly formed phloem within the wood. In other forms, of which *Strychnos* is the best known example, the included phloem strands are originally formed by the cambium towards the outside as a part of the normal external phloem but later on new " extra-fascicular " cambial segments arise in the pericycle and unite with the general cambium cylinder so that each phloem strand becomes enclosed between a segment of the old or " residual " cambium and the new one. Of these the former becomes more or less inactive, but the latter produces xylem and phloem in the normal way so as to bury the included phloem deeper and deeper into the stem. This process is repeated several times at a number of places all around the circumference of the stem resulting in numerous " islands " of phloem within the xylem. Eames and MacDaniels (1925, p. 258) further remark that the behaviour of the cambium in such growth types has been studied in detail in but a few instances and add that probably only one of these methods (the *Strychnos*-type) occurs in all cases.

As regards *Salvadora persica*, which is the subject of the present study, Rosenvinge (1880) thought that the phloem islands (see Fig. 1) were cut off centripetally by the first method. Scott and Brebner (1889) and Chodat (1892) supported this view. A few years later Leisering (1899) ' from an examination of dozens of preparations

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[1] In accordance with the terminology given by Record (1933, p. 2) the term " Included phloem " is used in this paper in place of the former " Interxylary phloem ".

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of the stem' found that the phloem of the mature islands is not in direct contact with the xylem on its inner side (which ought to be the case if Rosenvinge's view is correct), but is separated from the latter by at least one and usually 2–3 layers of cambial cells. He there-fore concluded that the development is similar to that in *Strychnos*, although he could not prove it with absolute certainty. Pfeiffer (1926) calls attention to the controversy regarding the origin of the phloem islands in *Salvadora* and suggests the need for a reinvestigation.

2. MATERIAL AND METHODS

Salvadora persica is a common tree in the North, West and South India. The material used in this study was collected from Agra, partly by Dr. P. Maheshwari and partly by Mr. B. L. Gupta and consisted of pieces of the root and stem preserved in formalin-acetic-alcohol. As the woody portion is very hard, short pieces of the stem and root were treated with dilute HF before embedding them in paraffin. Some sections were also cut on the sliding microtome without any embedding or pre-treatment. Safranin and Fast Green were used for staining. Some material of *Salvadora oleoides*, collected from Bharatpur, was also cut for comparison.

3. THE STEM

General Anatomy.—In a t. s. of a moderately old stem the epidermis is seen to be replaced by a superficial layer of cork, whose outer cells seem to flake off at intervals (see Fig. 2). The phellogen arises sub-epidermally and cuts off centripetally a narrow zone of tangentially elongated cells composing the phelloderm.

The cortex is narrow consisting of only a few layers of thin-walled cells often containing druses or rhomboidal crystals. Due to the secondary changes in the stem these cells have a more or less flattened and distorted outline. An endodermis is not clearly differ-entiated, but the beginning of the pericycle is indicated by isolated groups of sclerenchymatous fibres connected to one another by means of thick-walled pitted cells.

At this stage the primary phloem is already crushed and almost indistinguishable. Even the secondary phloem is a narrow zone (Fig. 2), but this deficiency is more than made up by the intra-cambial phloem "islands" which are numerous and form a very conspicuous feature of the stem.

Next to the phloem is the cambium cylinder which is an actively dividing zone consisting of 4–5 layers of cells in my material. I am unable to support Leisering's statement that it forms a wide meriste-matic zone consisting of several layers of cells. He evidently included under the "cambium" the patches of undifferentiated parenchymatous cells cut off by it towards the inside which remain unlignified and even continue some periclinal divisions for a time (Fig. 1). It is these patches of thin-walled cells which subsequently become differentiated into the phloem islands which are dealt with in a subsequent paragraph. The wood forms the largest part of the stem. It consists of vessels,

parenchyma, and fibres. At the inner end are seen more or less radial rows of the first formed xylem vessels which protrude into the pith. Some of the protoxylem vessels are seen to be distorted and crushed owing to the enlargement of the adjacent cells of the pith. In the secondary xylem the vessel elements are numerous, rather short and narrow-lumened and occurring in groups of 2 to 8. They have simple perforations and the walls bear many alternate bordered pits. The xylem fibres are numerous and thick-walled with a few reduced bordered pits having extended slit-like apertures. The xylem paren-chyma cells are particularly numerous around the vessels and phloem islands. Their walls are thin and have simple oval or rounded pits. The vascular rays are 1–7 cells wide and consist of radially elongated cells often containing squarish, rhomboidal or rectangular crystals. Such portions of the rays which pass through the phloem (normal or included) are thin-walled, but the rest consist of thick-walled pitted cells.

The pith has a more or less four-sided outline in a t.s. of a young stem. Its cells are spherical with pitted walls separated by small intercellular spaces ; some of them may contain solitary druses.

Origin and Structure cf the Included Phloem.—Soon after secondary growth has commenced, the outer margin of the secondary xylem presents a more or less indented outline, which is due to the fact that at certain places the cambium cuts off towards the inside some tangentially elongated groups of thin-walled parenchyma in place of the usual lignified cells of the wood. These cells lie in perfect radial rows and differ from the cambial cells, from which they have been produced, only in having a larger radial diameter. After a short while the cambium resumes its normal activity so that the bays of thin-walled parenchyma become enclosed between the thick-walled cells of the previous and the newly formed wood. As growth proceeds some of the central cells in the islands differentiate into one or more groups of sieve tubes and companion cells (Fig. 3) but the peripheral cells still remain parenchymatous and often undergo some periclinal divisions so as to form a weak secondary cambium on the inner as well as the outer face of the phloem island (Fig. 4). In some cases the differentiation of phloem elements in the island may start even before it is actually buried into the wood, so that for a short while and at certain places phloem cells may be seen on both sides of the cambium cylinder. Only the outer of these belong to the normal phloem which is however extremely reduced in quantity, while the inner are destined to become included in the xylem. The sieve plates are usually obliquely placed. There is no special structural difference between the outer and the included phloem except that in the former some thick-walled fibres are also present. In a t.s. of a stem, about 1·3 cm. in diameter, more than 600 " islands " were counted in the wood and since *Salvadora persica* is a fairly large tree, their number must run to several thousands at the base of the stem. The inner and earlier formed phloem islands are smaller than the outer. They are further more or less circular or oval, while the outer and more recently formed groups of phloem

are very much elongated tangentially, some even becoming confluent with one another at the sides.

In the older and consequently more deep-seated islands some of the central cells get degenerated and form one or more darkly staining patches in each island (Fig. 5). As regards the cause for this obliteration Pfeiffer (1926) was unable to come to a decision and suggested two possibilities, *viz.*, that this condition may either be due to a continued activity of the primary cambium enclosed in the bundle (as in *Strychnos*) or it may be caused by a division of the elements composing the island itself. My observations lead me to the conclusion that the second view is the correct one for *Salvadora*, as also for *Leptadenia* (Singh, 1943). Cell divisions accompanied by subsequent differentiation and enlargement of the elements continue to take place in the islands for a fairly long time, but being enclosed by the thick-walled cells of the wood on all sides, the phloem cells do not find enough space for outward expansion and the whole force is therefore directed inwards resulting in a crushing of the central cells. It is also observed that the thin-walled cells of the rays which happen to pass through the older islands may similarly get crushed along with the phloem elements.

As noted before, there are often seen on the margins of the older islands a few cells which have a cambial nature. A careful study revealed that this is a secondary meristem which may appear in segments on any one side, or more than one side of the island or even all round it. If it first arises on the inner side it may give the false impression that it is an embedded segment of the original cambium ring and that the development is of the *Strychnos*-type. The activity of this cambium-like layer, and the pressure caused by it towards the centre of the island, may also be partly responsible for the crushing of cells in this region.

4. THE LEAF

Petiole.—A t.s. of the petiole has a more or less circular outline with the upper side slightly flattened. The epidermis is heavily cutinised on the outside. The cortex is a broad zone of thin-walled parenchymatous cells having small intercellular spaces between them. The outer cells, forming a 2–3 layered hypodermis are polygonal, with slight thickenings at their angles and often without any intercellular spaces. There is no indication of an endodermis. The stele has an oval or flattened horse-shoe shaped form with a much greater development of the vascular tissue towards the rounded abaxial side. Small groups of fibres lie outside the isolated groups of crushed primary phloem cells. In the centre there is a very narrow flattened pith consisting of small thin-walled cells. The structure of the xylem and phloem is somewhat similar to that in the stem and it is worthy of note that the included phloem is present even in the xylem of the petiole.

Lamina.—The upper and the lower epidermis each consists of 1–2 layers of cells. Their outer walls are covered with a thin layer

of cuticle. Stomata are present on both sides. The guard cells are small and are somewhat sunken below the general level of the epidermal cells. Subsidiary cells lying parallel to the stomatal pore are clearly seen in surface preparations. The mesophyll consists of a palisade tissue on both sides enclosing a parenchymatous region along the middle line. The palisade cells are tubular and compactly packed and contain a large number of chloroplasts whose number gradually diminishes towards the interior. Interspersed amongst them are some large pouch-like cells which are considered by Sabnis (1921) to have a water-storage function but it seems that he did not notice the large spheroidal crystal in each of these cells which usually dissolves in the process of preparation of the slides. It may be added that Sabnis also failed to observe the crystals in the inner tissues of the leaf in either *S. persica* or *S. oleoides*. The cells between the two palisade regions are horizontally elongated and contain very few or no chloroplasts. Here and there in this region occur the veins and some small groups of thick-walled pitted cells which may either serve for storage of water or merely serve to give the necessary rigidity to the leaf.

The *midrib* shows a greater convexity towards the lower side. The palisade cells are continued on the upper side, but their place is occupied on the lower by large collenchymatous cells which may or may not have any intercellular spaces between them. The included phloem continues for a certain distance in the lamina, but as the amount of secondary growth decreases, it gradually disappears towards the tip.

5. The Root

General Anatomy.—The root may be diarch to tetrarch. The primary xylem strands frequently meet in the centre to form a solid xylem plate but more often a small pith is present. Secondary growth begins early and is accompanied by cork formation. The primary phloem soon becomes crushed and is unrecognisable. The secondary phloem occurs in the form of pyramidal strands composed of sieve tubes, companion cells, fibres and phloem parenchyma. In older roots it is the secondary xylem which occupies the largest proportion of the space in a t.s. Like the stem it also contains a considerable number of phloem islands scattered through it. The wood elements consist of vessels, fibres and parenchyma. There are two to four primary rays, one opposite to each primary xylem group. These are much wider than the subsequently differentiated secondary vascular rays which have the same structure as those in the stem.

Origin and Structure of Included Phloem.—The phloem islands arise in the same way as in the stem. The later formed islands are usually larger and more elongated than the first ones. Although they do not occupy any definite position in the wood, it appears that at least in later stages there is some kind of a periodicity in the activity of the cambium, which, roughly speaking, produces the thick-walled cells destined to form the wood and the thin-walled cells destined to form the included phloem, in more or less alternating layers.

The structure of the individual islands, the course of degeneration of the phloem cells and the differentiation of the secondary cambium show such close similarity with the stem that a detailed description seems unnecessary.

6. COMPARISON WITH *Strychnos nux-vomica*

While studying the origin of the phloem islands in *Salvadora*, I also cut for comparison some material of *Strychnos nux-vomica*[2] in order to gain a better insight regarding the resemblances and differences between these two forms.

My observations on *Strychnos* fully confirm those of Scott and Brebner (1889) whose account has for a long time remained the only clear exposition of the origin and nature of the phloem islands. As stated by them the islands are at first produced centrifugally as a part of the normal external phloem, but later they become bridged over by the " complementary " cambial segments, which join on to the main cambial ring (Fig. 6). These short cambial arches function normally by producing xylem towards the inside and phloem towards the outside. As a result the first formed phloem groups become buried into the wood, each having a centripetally embedded cambial segment which was once a part of the original cambium. This continues to cut off some new phloem towards the outside (but no xylem) with the result that the older phloem cells become crushed to form a sort of a cap on the outer face of each island. The islands in this species of *Strychnos* are small and circular or oval but in *Strychnos Mitscherlichii* Cockrell (1941) found that they are tangentially elongated.

It is to be noted that in *Salvadora* the condition is quite different. Unlike *Strychnos nux-vomica* the included phloem is here cut off centripetally from the normal cambial cylinder. The islands do not have any embedded cambium that can be traced back to the original cambium ring but a weak secondary cambium may occasionally arise on one or more than one side of some of the older islands. Further, the crushing of the phloem tissue takes place in the centre and not on the outside. The cap-like tissue of disorganised cells, seen in *Strychnos* is therefore not found here.

7. CONCLUSION AND SUMMARY

In conclusion, it may be said that the included phloem found in the wood of *Salvadora* (root as well as stem) is differentiated from the thin-walled parenchymatous cells cut off by the cambium on its inner side. Subsequently the cambium resumes its normal activity and the phloem becomes more and more deeply embedded into the wood.

If Leisering (1899) had made a developmental study of the included phloem and also compared the position of the crushed cells in the islands in *Strychnos* and *Salvadora*, he would not have come to the conclusion that they have a similar origin. As shown in this paper

[2] The material of this plant was very kindly collected for me by Mr. J. Venkateswarlu (Waltair) and Mr. M. A. K. Khalil (Dehra Dun).

it is not satisfactory to rely on the presence or absence of an embedded cambial segment on the inside of an island, for such a cambium although it seems to be characteristic of the *Strychnos*-type, may differentiate secondarily in other cases.[3] It may be pointed out that the suggestion (Eames and MacDaniels, 1925, p. 258) that of the two methods of origin of included phloem, only the *Strychnos*-type occurs in all cases and the other is probably non-existent, is incorrect.

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[3] Besides *Salvadora* which has been described here and *Leptadenia* (Singh, 1943), such a secondary meristem is also reported to occur around certain groups of phloem cells in the roots of some Cruciferoe (Weiss, 1883), Cucurbitaceae (Scott and Brebner, 1889), *Ipomoea batatas* (Artschwager, 1924), and *Asclepias obtusifolia* (Scott and Brebner, 1890-91).

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                                 390-97, 401-15.

10. EXPLANATION OF FIGURES

PLATE III

Fig. 1. *Salvadora persica.*—A cross-section of an old stem to show the distribution of the phloem islands. × 5·5.

Fig. 2. *S. persica.*—A segment of an old stem showing the origin of the included phloem. × 66.

Fig. 3. *S. persica.*—A young phloem island showing the differentiation of sieve tubes and companion cells. × 180.

PLATE IV

Fig. 4. *S. persica.*—An included phloem island with some cambium-like cells on its outer as well as inner side. × 410.

Fig. 5. *S. persica.*—An old phloem island showing the crushing of the central cells. × 410.

Fig. 6. *Strychnos nux-vomica.*—Portion of cross-section of a stem showing the centrifugally formed phloem with an extrafascicular cambial segment on its outer side. × 290.

Fig. 1
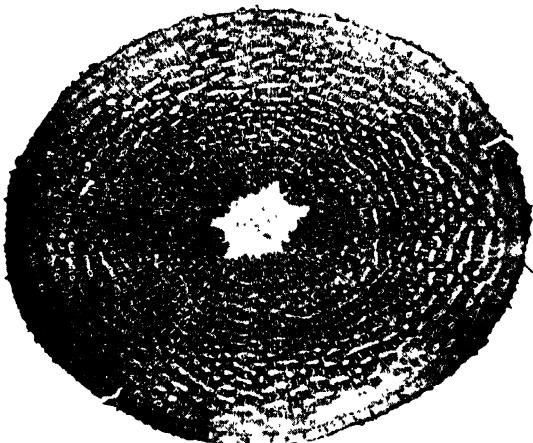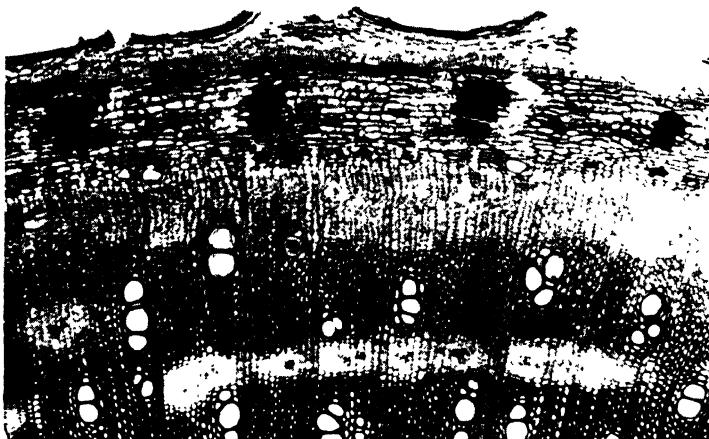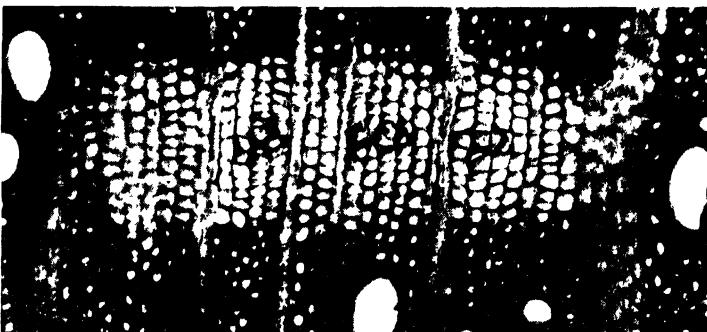

Fig. 2

Fig. 3

BALWANT SINGH

A CONTRIBUTION TO THE ANATOMY OF SALVADORA PERSICA L.
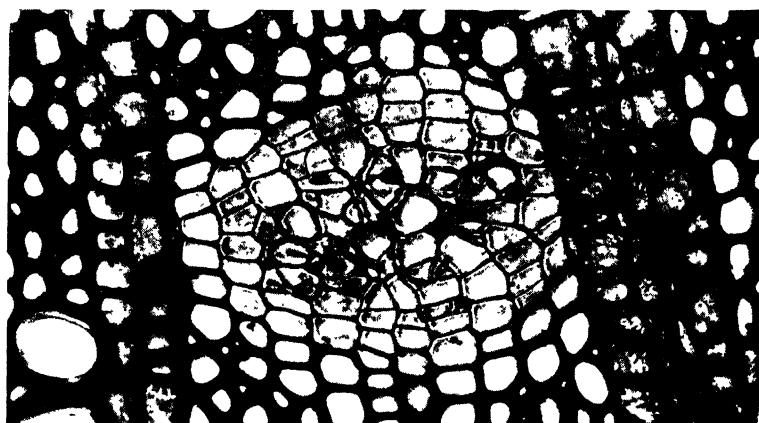
FIG. 4
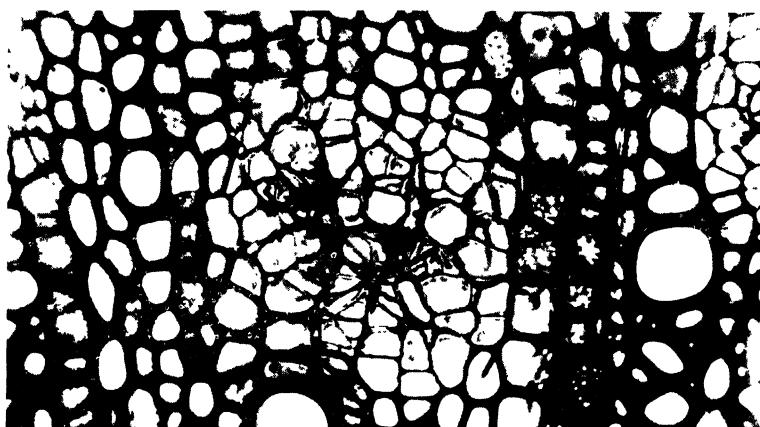
FIG 5

F G. 6

BALWANT SINGH—

A CONTRIBUTION TO THE ANATOMY OF SALVADORA PERSICA L.

THE ORIGIN OF THE HAUSTORIA IN THE OVULE OF *LOBELIA*

By P. Maheshwari

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DR. G. O. COOPER (1942) has recently published a paper on the embryology of *Lobelia cardinalis* L. in which it is stated that the synergids and antipodal cells function as micropylar and chalazal haustoria respectively.[1] The only recent work on the Lobeliaceæ and Campanulaceæ, referred to by him, is that of Kausik (1938) on *Lobelia nicotianaefolia*. There is no mention whatever of the works of Rosén (1932), Kausik (1935), Hewitt (1939) and others.

Kausik (1935, 1938) and Hewitt (1939) report that the synergids and antipodal cells in *Lobelia* degenerate at the time of fertilisation and this is in agreement with the observations made by Rosén (1932) and Safijovska (1934) on the allied family Campanulaceæ.[2] There is thus no possibility of their being responsible for the haustorial outgrowths which are clearly endospermal. In the absence of any material of *L. cardinalis* and the improbability of my being able to get it for the duration of the war, I requested Dr. S. B. Kausik of Bangalore for the loan of his preparations of *L. trigona* so that I might make an independent study of them in the light of Dr. Cooper's observations. The result of this study confirms Dr. Kausik's interpretation that the haustoria (both micropylar and chalazal) originate from the endosperm and have nothing to do with the synergids or the antipodal cells which degenerate at the time of fertilisation or shortly afterwards.

It may be added that really haustorial synergids are probably known only in a few Compositæ but even there a detailed and illustrated account of their development has never been published up to this time. In all other cases the occurrence of synergid haustoria (for a meaning of the term " haustorium " see Schnarf, 1929, pp. 352–55) is extremely doubtful. To mention a parallel instance, Heinricher (1931/32), in his monograph on the genus *Lathræa*, stated that the micropylar haustoria are derived from the synergids and the chalazal from the antipodals. This was promptly contradicted and disproved by Glišić (1932) wao made a thorough study of *Lathræa squamaria* and found that the haustoria are derived from the endosperm. The report of V. K. Srinivasan (1940) on the persistent and

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[1] In the original (Cooper, p. 81) the order is given as " chalazal and micropylar " but this must be an oversight.

[2] Some authors include both the families (Lobeliaceæ and Campanulaceæ) under a common name.

4

presumably haustorial synergids of *Angelonia* has already been criticised by Maheshwari and Navalakha (1941) and more recently by Dr. C. V. K. Iyengar (1942). Two similar looking structures seen in *Myriophyllum alterniflorum* have been shown to be derived by a vertical division of the basal cell of the suspensor (Stolt, 1928). It is noteworthy that in this case these two cells develop even the " filiform apparatus " and hooks found in genuine synergids and the similarity is so deceptive as to mislead even the most cautious unless he has taken pains to obtain a complete series of stages in the development.

Further, it is to be noted that although one would expect *three* chalazal haustoria (granting their antipodal origin) in *Lobelia cardinalis*, actually only *two* are present and to explain this Dr. Cooper (p. 77) says that the third antipodal cell becomes " appressed " owing to the growth of the endospermal cells. Again, although double fertilisation is figured and said to take place normally there is no trace of the pollen tube in any of Cooper's figures either at the time of fertilisation or after it. On the other hand, in Kausik's (1935) figures of *L. trigona* and Hewitt's (1939) of *L. amoena* the pollen tube is quite clear and unmistakable. One would like to know how the synergids react to the pollen tube in *L. cardinalis* since in all other plants of the Lòbeliaceæ and Campanuliceæ, at least one or both of them begin to disorganise on its impact.

I wish to thank Dr. Kausik for the loan of his preparations of *Lobelia trigona*. Some material of *Wahlenbergia gracilis* and *Sphenoclea zeylanica* (Campanuliceæ) as well as *Lobelia trigona*, which I recently collected from Dacca, also shows that the antipodal cells and synergids are ephemeral and the haustoria are formed from the endosperm. A more detailed account of the embryology of these plants particularly with reference to the haustoria (on whose exact origin from the endosperm there seems to be no agreement), will follow in due course, but meanwhile it is suggested that Dr. Cooper may re-examine his preparations of *L. cardinalis* in the light of the above remarks.

SUMMARY

From a comparison of Dr. G. O. Cooper's work on *Lobelia cardinalis* with the figures and descriptions of other workers on the embryology of the Lobeliaceæ and Campanuliceæ, there seems to be no doubt that the haustorial structures which Dr. Cooper believes to have been derived from the synergids and antipodal cells are really formed from the terminal portions of the endosperm cells—a condition which is of wide occurrence in the Sympetalæ.

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DADOXYLON RESINOSUM SP. NOV. FROM THE CHHINDWARA DISTRICT OF THE CENTRAL PROVINCES

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THE block of gymnospermous wood here described belongs to the Central Museum, Nagpur. It is a solitary specimen of its kind and had been lying in the museum since the time of the late Rev. S. Hislop. The entries in the museum register indicate that Hislop had collected the fossil in the Chhindwara district but the exact locality is unknown. Nothing definite can therefore be said about the geological age of the specimen and it may be safer to discuss it separately.

Dadoxylon resinosum sp. nov.

(Figs. 1–21)

Diagnosis.—Growth rings well marked. Resiniferous tracheids mixed with medullary rays. Wood parenchyma absent. Tangential pits 1–2 seriate, bordered, isolated, round, sometimes alternate, contiguous and hexagonal due to contact. Radial pits 1–4 seriate, separate and circular or contiguous and hexagonal, sometimes opposite. Pore usually circular, large. Rims of Sanio absent. Medullary rays 1–2 seriate, 1–39 cells high, average height 22 cells. End walls of the medullary rays mostly transverse, sometimes oblique. Pits in the field 1–10 (generally 4–6), simple, usually round.

Locality.—Chhindwara district, C.P., India (exact locality unknown).

Horizon.—Unknown.

Collected by.—S. Hislop.

Reg. No.—F/149, Central Museum, Nagpur, C.P., where the type specimen is preserved.

V. B. SHUKLA

DESCRIPTION

The specimen is a fairly well preserved block of secondary wood, 13 cm. long and 3·5 cm. ×3 cm. across the larger end (Fig. 10). The growth rings being very slightly arched (Fig. 1), the wood appears to be a piece quite far away from the pith. The autumn wood appears
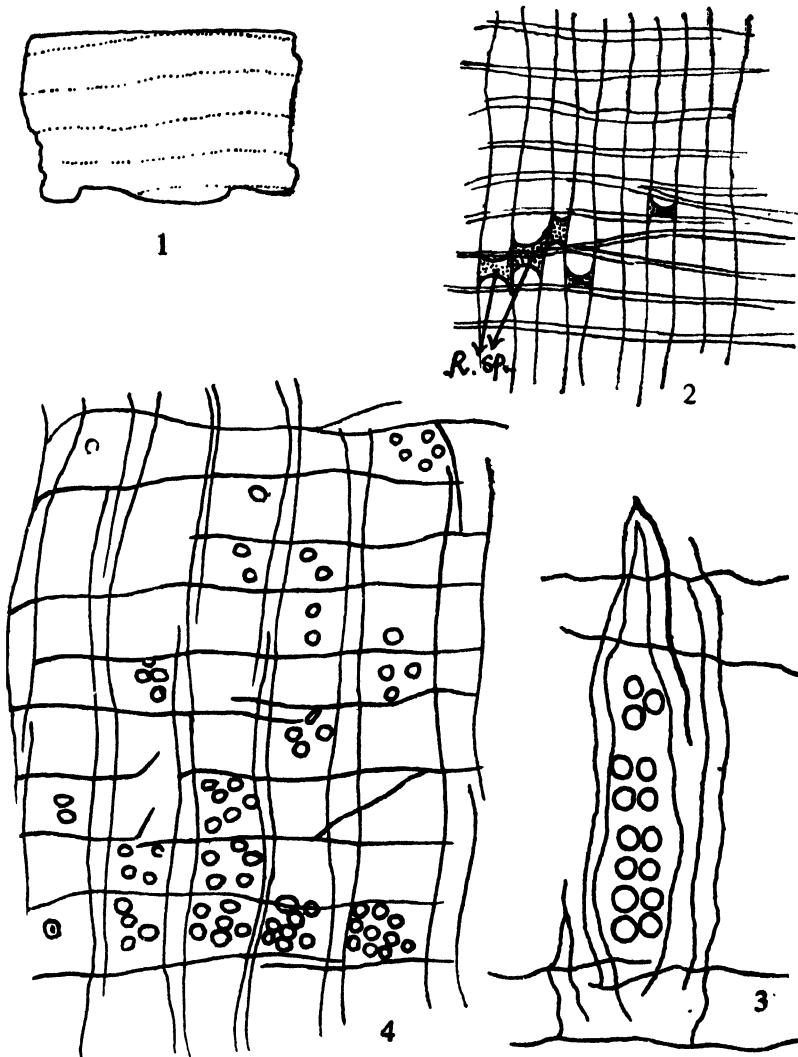
Figs. 1—4. *Dadoxylon resinosum.*—Fig. 1. Type specimen ; transverse view of the stem showing growth rings. × 1. Fig. 2. Radial section. *R.Sp.,* resin spools in the medullary rays. × 100. Fig. 3. Radial section showing a single tracheid having biseriate opposite pits only. × 200. Fig. 4. Radial section showing pits in the field. × 250.

like a thin brown line about ·2 mm. thick, the spring zone being on an average 5 mm. wide.

In transverse section the growth rings are visible macroscopically (Fig. 1) as well as microscopically (Fig. 11). The autumn zone is usually represented by three or four narrow layers of tracheids (Fig. 12). The tracheids as a rule are thick walled (average ·02 mm.) and those of the spring zone are usually isodiametric. Some of the tracheids are plugged with black stuff (? resin). The medullary rays are very crowded, being often separated by only one or two rows of tracheids. Some of the rays are also filled up with black resinous looking stuff and that gives them a prominent black appearance (Figs. 11 and 15).

The medullary rays are generally uni- or biseriate (Figs. 5 and 13). The biseriate portion is not necessarily in the middle but at or near the ends (Fig. 5, *a, b*) ; or portions of one ray may be alternately uni- and biseriate (Fig. 5, *d*). In some cases the medullary rays appear to be forked at the tips (Fig. 5, *c*). Their height is 1–39 cells (average 22 cells).

Tangential pits 1–2 seriate, usually round and solitary (Figs. 2 and 14), in some cases contiguous hexagonal.

The end walls of the medullary rays are mostly transverse, sometimes oblique. The resiniferous tracheids may be solitary or in groups of several (Fig. 11). The resin in most of the tracheids plugs the whole width of the cavity and sometimes it fills a fair length of the tracheid ; it always takes the form of a solid black substance (Fig. 15).

Resin plugs are also seen in some of the medullary rays (Figs. 2 and 15, *R. Sp.*).

The radial pits are 1–4 seriate. The uniseriate (Fig. 17) and tetraseriate conditions (Fig. 7) are rare, the triseriate (Figs. 9 and 19) is occasional and the biseriate (Fig. 16) is the commonest. The pits may be contiguous in separate rows (Fig. 16) ; mixed uni- and biseriate conditions also occur (Fig. 18).

The pits show a tendency to lie alternately when in two or more rows (Figs. 8, 16 and 19) and in some cases within the same tracheid there may occur contiguous hexagonal and also opposite pits (Figs. 8 and 9 ; opposite pits are arrow marked). Several tracheids contain exclusively contiguous and hexagonal pits (Fig. 20).

The pits in certain tracheids are exclusively opposite (Fig. 3).

The pits in the field, 1–10 in number (Figs. 4 and 21), are mostly simple.

Average radial diameter of the widest spring tracheid (50 counts)	·066 mm.
Avera e radial diameter of the narrowest autumn tracheid	-·018 mm.
Height of a vertical row of ten radial pits .	·11 mm.
Height of the medullary rays	- 22 cells.
Dimensions of the medullary ray cells . ..	·027 × ·033 mm.
Thickness of the tracheid wall	= ·027 mm.
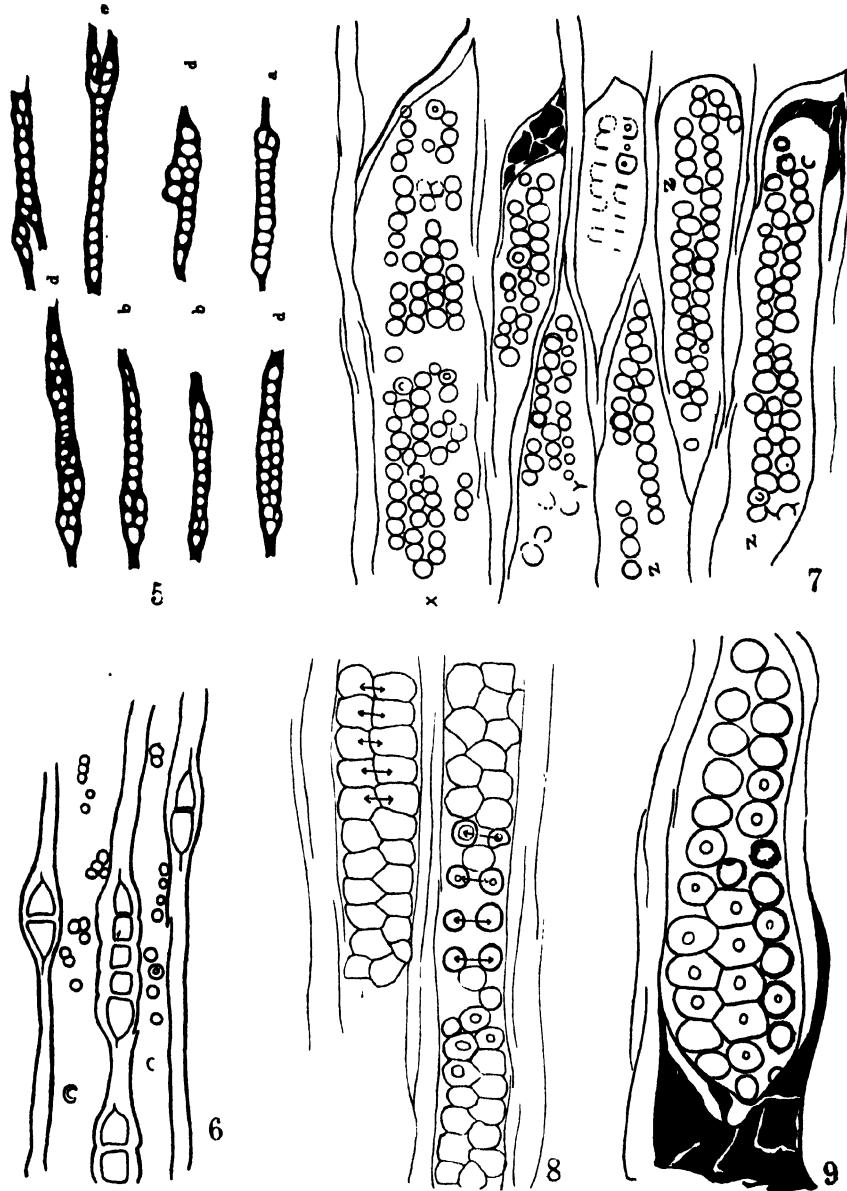
Figs. 5—9. *Dadoxylon resinosum.* –Fig. 5. Various types of medullary rays as seen in tangential section. (For explanation of *a, b, c* and *d* see text.) × 38. Fig. 6. Part of a tangential section showing pits. × 60. Fig. 7. Radial section showing arrangement of bordered pits. × 150. Fig. 8. Two tracheids from a radial section having both contiguous and opposite pits. × 150. Fig. 9. A single tracheid from a radial section having triseriate contiguous hexagonal as well as biseriate opposite pits. × 225.

Systematic Position and Comparisons

As suggested by Prof. Seward (1917, pp. 249–50), the term *Dadoxylon* is used for all gymnospermous woods showing contiguous hexagonal pitting whether they are Cordaitean or Araucarian ; hence the present wood is assigned to that genus. But as the age is quite unknown, it is impossible to say whether it is a Cordaitean or an Araucarian wood. It has been compared with all the southern species of *Dadoxylon* and with *Dadoxylon (Mesopitys) Tchihatcheffi,* the solitary species showing growth rings amongst the northern forms. In the following table only those characters are given in which the various species named therein differ from *D. resinosum.*

It is thus observed that the present wood differs from all the known species of *Dadoxylon.* The maximum resemblance is with *Dadoxylon Deccani* Shukla, particularly in the presence of opposite pits mixed up with contiguous hexagonal pitting ; but the characters of the medullary rays, the pits on the tangential walls of the tracheids, the 2–4 seriate nature of the pits and the thick walled tracheids are enough to distinguish it. This wood, hence, appears to be a new species and it is proposed to name it as *Dadoxylon resinosum* sp. nov., the specific name referring to the abundance of resin-like substance in the tracheids.

Discussion

From the literature available on the geology of the Chhindwara district, it is gathered[1] that the only sedimentary beds found within the district are (*a*) the Lametas, (*b*) the Gondwanas, and (*c*) the inter-trappeans. On the whole it seems more likely that our fossil comes from the Intertrappean series which is well known to have yielded a rich flora than that it belongs either to the Lametas or the Gondwanas of the district from which we at present know hardly any recognisable fossil woods. The Intertrappean series is now considered to be Eocene.[2]

It may, however, also be mentioned that during the Eocene age it was the Araucarian members of the artificial genus *Dadoxylon* that had persisted and not the Cordaiteans and thus the present wood may more appropriately be designated as *Dadoxylon (Araucarioxylon) resinosum* sp. nov.

Next comes the question of the systematic position of this fossil and in this connection it may be said that the occurrence of both alternate and opposite pits may be interpreted in the same way as in *Dadoxylon Deccani* Shukla. This wood may hence be considered as another connecting link between the families Araucarineæ and Abietineæ.

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[1] Medlicott, H. B. and Blandford, W. T. (1893), pp. 92–167.

[2] Sahni, B. (1934), pp. 134–136.

TABLE I.

	Medullary rays	Other characters
Dadoxylon (Mesopitys) Tchihatcheffi[1]	Markedly low	1-5 rows of pits. Field pits without a border.
D. Zalesskyi Sahni[2]	Uniseriate, low	Rims of Sanio present.
D. Krauseli Sahni & Singh[3]	1–20 cells high	Secretory pits present.
D. lafoniense Halle[4]	Low 1-ser., pits 2-ser.	1–2 rows of pits. Pits having a tendency to fuse and unite to form a single big pit.
D. indicum Holden[5]	Low, uniseriate	
D. bengalense Holden[6]	Uniseriate, 1–20 cells high	2–7 field pits forming a group.
D. (Araucarioxylon) rajmahalense Sahni[7]	Uniseriate, 1–20 cells high	2–3 seriate pits.
D. α Sahni[8]	Uniseriate, low	
D. β Sahni[9]	Uniseriate, low	Pits 2–3 seriate. Growth rings not well marked.
D. parbeliense Rao[10]	1–24 cells high	Pits 1–5 seriate. Field pits 8–9.
D. teilhardi Sze[11]	1–6 cells high	Biseriate pits. Field pits 8–9.
D. rhodeanum Göppert[12]	3–20 cells high	2–4 rows of pits. Annual rings absent.
D. Deccani Shukla[13]	Mostly uniseriate, 2–49 cells high	1–2 seriate pits. Field pits 1–6. No tangential pits.
D. Bakeri Seward & Walton[14]	1–16 cells high	
D. sp. Warren[15]	1–20 cells high	
D. sp. Walton[16]	1–20 cells high	
D. angustum Felix[17]	1–25 cells high	
D. Arberi Walton[18]	1–25 cells high	

[1] Frentzen, K. (1931), pp. 617-24.
[2] Sahni, B. (1933), p. 421.
[3] Sahni, B. and Singh, T. C. N. (1926), pp. 103-12.
[4] Halle, T. G. (1911), p. 61.
[5] Holden, R. (1916), p. 318.
[6] Holden, R. (1916), p. 322.
[7, 8, 9] Sahni, B. (1931), pp. 69, 71, 72 respectively.
[10] Rao, H. S. (1936), p. 174.

[11] Sze, H. C. (1934).
[12] Gothan, W., und Sze, H. C. (1933), pp. 87–103.
[13] Shukla, V. B. (1938), pp. 359–62.
[14] Seward, A. C., and Walton, J. (1923), pp. 313–33.
[15] Warren, E. (1912).
[16] Walton, J. (1925), p. 2.
[17] Halle, T. G. (1911), p. 68.
[18] Walton, J. (1925), n. 2.

SUMMARY

The present wood is the second petrified gymnospermous wood to be described from the Chhindwara district. The exact locality and therefore the geological age is unknown. It is, however, quite possible that this wood might have come from the Intertrappean beds of that district, which are 'now considered to be of Eocene age. Comparisons have been made with all the known *Dadoxyla* of the Gondwana type, including *Dadoxylon* (*Mesopitys*) *Tchihatcheffi*, but as it differs from all of them, it has been referred to a new species *D. resinosum*. The specific name refers to the abundance of a resin-like substance in the tracheids which seems to be a characteristic feature of the wood. As this species, like *D. Deccani* Shukla, shows a combination of alternate and opposite pits, it may also be considered as a connecting link between the families Araucarineæ and Abietineæ.

ACKNOWLEDGMENT

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EXPLANATION OF PLATES

Plate V

Fig. 10.   *Dadoxylon resinosum* sp. nov. Type specimen ($\times$ 9/13).

Fig. 11.   Type specimen. Transverse section showing a growth ring ($\times$ 30)

Fig. 12.   Type specimen. Transverse section showing an autumn zone and parts of two spring zones on the either side ($\times$ 200).

Fig. 13.   Type specimen. Tangential section showing uni- and biseriate medullary rays ($\times$ 100).

Fig. 14.   Type specimen. Tangential section showing bordered pits on the tangential walls of the tracheids ($\times$ 290).

Plate VI

Fig 15.   Type specimen. Radial section showing tracheids and medullary rays plugged with condensed black resiniferous substance ($\times$ 15 0)

Fig. 16.   Type specimen. Radial section showing biseriate condition of pits in the tracheids ($\times$ 230).

Fig. 17.   Type specimen. Radial section showing uniseriate condition of pits in the tracheids ($\times$ 130).

Fig. 18.   Type specimen. Radial section showing tracheids with uni- and biseriate pits in the same trracheid ($\times$ 150).

Fig. 19.   Type specimen. Radial section showing a tracheid with triseriate pits ($\times$ 230).

Fig. 20.   Type specimen. Radial section showing tracheids with contiguous hexagonal pitting ($\times$ 230).

Fig. 21.   Type specimen. Radial section showing pits in the field ($\times$ 410).

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V. B. SHUKLA—*DADOXYLON RESINOSUM* SP. NOV.
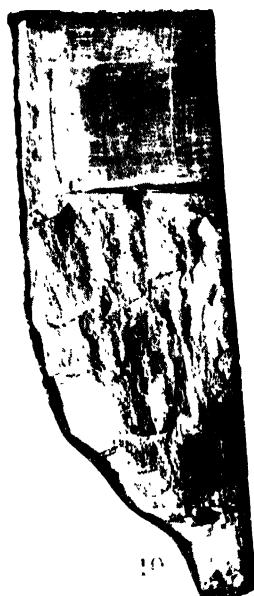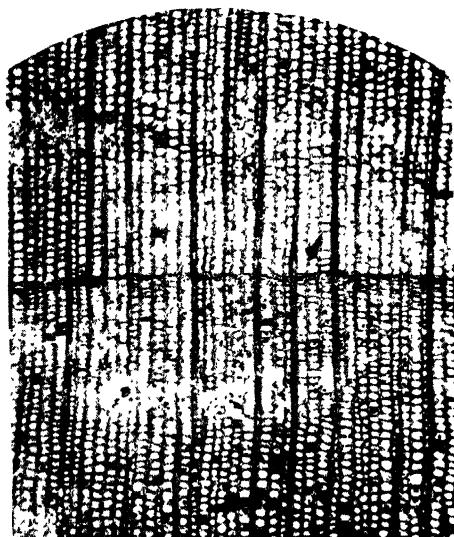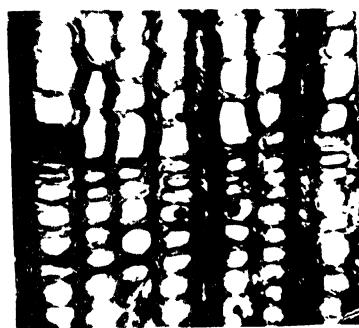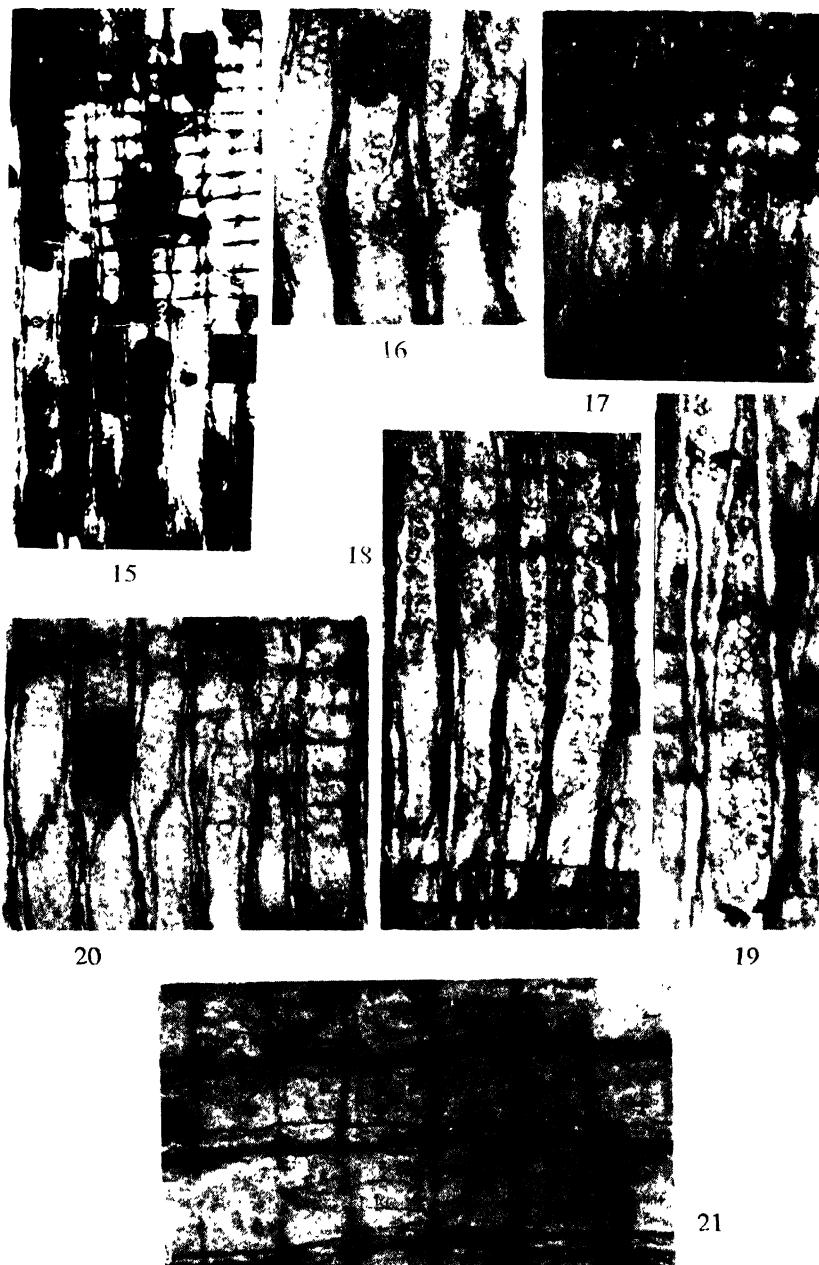
PHYSIOLOGY OF *CERCOSPORA SESAMI* ZIMM.

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I. INTRODUCTION

A LEAF SPOT disease of *til* (*Sesamum indicum*) caused by *Cercospora sesami* Zimm. has been prevalent in Sylhet and its neighbourhood for some years. It has been found to cause considerable damage. This disease has been known also to occur in other parts of Assam wherever *til* is grown and has also been reported from Bombay by Uppal, Patel and Kamat (1935).

In this paper are reported the characteristics of the disease and the results of the studies on the morphology and physiology of the fungus. These studies throw light on the role of external factors on the nature and spread of the disease.

II. SYMPTOMS OF THE DISEASE

The disease usually makes its appearance just at or before the time of flowering. But sometimes plants a month old have also been found attacked. The attack is more severe in the later stages.

The disease manifests itself generally as small light brown spots 2–5 mm. in diameter on the leaves. These spots are at first more or less roundish, but later on become irregular in outline and occasionally several coalesce forming irregular spots often as big as 5–15 mm. in diameter. The spots are found on both surfaces of the leaf. The colour of the spots which is at first light brown changes to a darker colour with the formation of the conidiophores and the conidia. The leaf tissue around the spots very often loses the normal green colour and takes a yellowish hue.

On the petiole the spots appear along its length ; they are elongated and of varying lengths. They are at first light brown as on the leaves but gradually become dark.

The stem as a rule is much less infected than the leaves. In shape and colour the spots on the stem resemble to a very great extent those on the petiole. Sometimes the whole stem dries and the plant droops down.

Lesions also appear on the pods and they are often numerous. They are more or less circular and measure from 1–7 mm. in diameter. In early stages the spots are brown in colour but in advanced stages they become black and often the pod is destroyed by the parasite. Fig. 1 shows the symptoms of the disease.

2

III. Morphology of the Parasite on the Host

Mycelium.—The mycelium of the fungus within the host is composed of irregularly septate thick-walled light brown hyphæ. Younger hyphæ are thin-walled and sparsely septate while the older ones are brown, thick-walled, tortuous with septa at short intervals. Mycelium is usually intercellular but in old, disintegrated tissues it also becomes intra-cellular. Hyphæ collect in the air spaces under the stomata and form stromatic masses giving rise to conidiophores which emerge through the stomata.

Conidiophores.—Conidiophores are light brown when young and dark brown when mature. They are produced in clusters, the number in each cluster varying from 5–10. They emerge through the stomata and clusters may be found to come out through most of the stomata. Each cluster has at its base a stromatoid structure (Fig. 2) made up of brown coloured cells. From these the conidiophores originate.
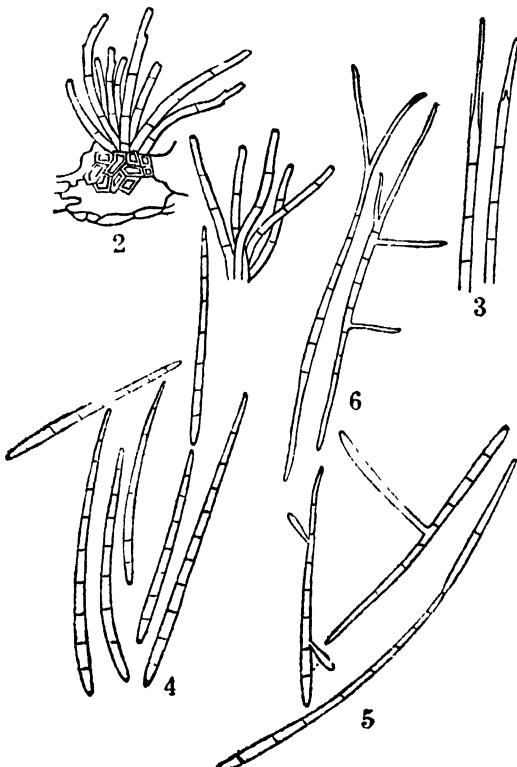
Figs. 2–6. *Cercospora sesami.* –Fig. 2. Conidiophores from nature. Fig. 3. Germination of conidiophores. Fig. 4. Conidia from nature. Fig. 5. Conidia giving rise to conidiophore and secondary conidia. 6. Germination of conidia. × about 600.

Each conidiophore is usully unbranched but branched conidiophores are by no means rare. They measure from $38 \cdot 5$ to $67 \cdot 5 \mu \times 4 \mu$ and are usually 0–3 septate. At the apex each conidiophore presents a number of characterisitc bends. At each bend there is a scar left by a spore. The scar appears as a slightly thickened area and looks darker under the microscope. The number of scars represents the number of spores produced and 1 to 6 scars may be noticed on a single conidiophore. The conidia are developed at the tips of conidiophores. After one conidium has been formed the stalk elongates past the conidium producing another again and thus the bends are formed. Conidiophores or their pieces readily germinate in tap water sending out germ-tubes from both ends as well as from sides near the septa (Fig. 3).

Conidia.—The conidia are sub-hyaline to very light yellowish, 7–10 septate and elongated, broader at the base and tapering towards the apex (Fig. 4). At the base of the conidium a scar is present showing the place of attachment to the stalk and this appears as a darker area. They measure $88–136 \mu$ in length and $3–4 \mu$ in width. The conidial wall is usually smooth but rarely constrictions are formed at the septa. Old conidia are of a brownish tint and sometimes one or two of their cells are found shrunken.

IV. PARASITISM

Single spore culture of the fungus was obtained by the usual plating method, and a series of inoculation experiments were carried out both in the field and the laboratory on plants of *Sesamum indicum* by placing spores and mycelium on unwounded host parts or by spraying with spore suspensions in sterile water. The results show that the fungus can attack every part of the host plant. The details of the inoculation experiments are summarised in Table I.

TABLE I

Summary of the results of inoculation experiments on
Sesamum indicum *by* Cercospora sesami

Parts of the host inoculated	No. of inoculations	No of plants infected	No. of controls kept	No. of controls infected
Leaves –				
Upper surface	86	71	39	Nil
Lower surface	78	62	42	,,
Petioles	27	25	22	,,
Stems	45	39	29	,,
Pods	48	41	32	,,

It will appear from Table I that the fungus is a parasite capable of attacking all parts of the plant. It was also observed that infection takes place readily both with mycelium and spores. In the case of mycelium as inocula infection takes place within 48 hours and with spore infection spots begin to appear after 4 to 6 days.

The fungus was isolated in all cases from the infected plants and it agreed with the parasite with which the infection was done.

V. STUDIES UNDER CONDITIONS OF ARTIFICIAL GROWTH

A. Macroscopic characters

(i) *Growth on different media*

The fungus was cultivated on a large number of media and on all the media the fungus made a good growth except on Brown's synthetic agar in which staling took place. The cultural characteristics of the fungal growth on the different media at 25° C. are recorded below :

Oat meal agar.—Abundant woolly aerial mycelium, colour white at the centre, light forgetmenot blue elsewhere and on the margin. Substratum light greyish indigo in the centre, edge light sky blue.

Dox's agar.—Abundant aerial mycelium slightly felty, dirty, very light green, edge light lilacy white. Substratum dark grey green in the centre, edge dark sheet grey.

Hopkins' agar.—Abundant woolly aerial mycelium, white to very pale yellowish, sparse on the edge. Substratum light cinnamon in the centre, then surrounded by a zone of dark blackish green.

Richards' solution agar.—Aerial mycelium profuse, woolly, light sky coloured white at places. Substratum dark cypress green.

Coons' agar.—Aerial mycelium abundant, cottony, very light purplish white, edge light greyish indigo. Substratum olive grreen.

(ii) *Depth of medium*

The effect of depth of medium upon linear rate of growth of the fungus was studied on Dox's agar and oat agar at 25° C. Petri dishes of equal size were supplied with 10, 20 and 40 c.c. of the media. Triplicate plates were used for each series. The inoculated plates were incubated at 25° C. and the diameter of the colonies was measured after seven and fourteen days. The details are given in Table II.

TABLE II

Influence of depth of medium on linear rate of growth of C. sesami

Media	Amount of media	Days	
		7	14
	c.c.	mm.	mm.
Dox agar	10	31·5	50·5
	20	35·5	57·0
	40	38·0	65·0
Oat agar	10	30·0	55·0
	20	31·5	60·9
	40	34·0	70·2

It will be obsreved from Table II that the linear rate increases with the increase in the depth and amount of nutrient. Similar results were obtained by Mitra (1931) on species of *Helminthosporium* and by Singh (1934) on *Cercospora indica* although Coons and Larmer (1930) found in the case of *Cercospora beticola* that the depth of media had very little influence on growth. It appears therefore that in carrying out determination of growth rate under different environmental conditions the depth of medium should be uniform.

(iii) *Light*

Effect of alternate light and darkness, continuous light from 100-watt electric lamp and continuous darkness on linear rate of growth was carried out on Hopkins' agar. To study the effect of alternate light and darkness inoculated plates were placed in front of a window at room temperature. Alongside of them cultures to be kept in continuous darkness were put inside big blackened cover dishes after wrapping them in black paper. Cultures to be kept in continuous light were placed in front of a 100-watt electric bulb in a darker corner of the same room. The diameters of the different colonies were measured after 10 and 20 days and the results are recorded in Table III.

TABLE III

Effect of alternate light and darkness, continuous light and continuous darkness on Hopkins' agar on the growth of C. sesami

	10 days	20 days
	mm.	mm.
Alternate light and darkness	22·0	34·4
Continuous darkness	18·2	24·0
Continuous light	12·6	18·3

It will be evident from Table III that the rate of linear growth is greater in alternate light and darkness, less in continuous darkness and least in continuous light. The retarding effect of continuous darkness and continuous light becomes more evident with time. Similar results were obtained with species of *Helminthosporium* by Mitra (1931).

(iv) *Humidity*

A study of the growth rate of the fungus under different relative humidities was made in accordance with the method of Stevens (1916). Cultures were exposed to atmosphere with different degrees of humidity by using sulphuric acid of varying specific gravities. Sterilized dishes of uniform size were used for the purpose and a known volume of the acid was put in each to fill about one-foruth of the volume. Petri dishes of uniform size in which equal amounts of the same medium had been poured and inoculated with the fungus were fixed with gelatine solution to glass panes big enough to fit on the top of the containers. The lids of the petri dishes were removed and the glass panes with dishes were sealed with vaseline to the containers

According to this arrangement the surface of the medium on which the fungus was growing was facing downwards exposed to the acid solutions, exerting known vapour pressure in the manner figured and described by Paul (1929). The rate of growth was measured and the data of the measurements, which are the average of two experiments, each running in triplicate, are given in Table IV.

TABLE IV

Linear rate of growth of C. sesami *in varying atmospheric humidity*

Relative humidity	Growth in millimeters		
	6th day	12th day	15th day
50 per cent.	24·0	26·1	27·4
70 ,, ...	22·6	28·5	31·9
78 ,, ...	19·8	32·7	36·0
92 ., ...	17·2	36·9	47·3
100 ., ...	12·9	25·2	36·0

The results presented in Table IV show that the fungus tolerates a wide range of humidity from 50–100 and the best growth is in an atmosphere of 92 per cent. humidity though the growth is faster at lower humidities during the first few days. In an atmosphere fully saturated with water vapour the growth is slow.

(v) *Temperature*

The linear rate of growth of *C. sesami* was studied on Hopkins' and Richards' solution agars at various temperatures. The experiment was carried out in selected Petri dishes of uniform size into which equal amounts of the medium were poured. All the dishes were inoculated at the same time and kept at various temperatures in darkness. The experiment was run in triplicate and repeated twice. The diameters of the growing colonies were measured from time to time and the data obtained are presented in Figs. 7, 8 and 9.
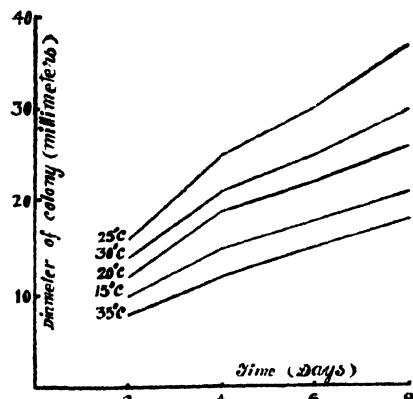
Fig. 7. Temperature relationship of *C. sesami* on Richard's solution agar
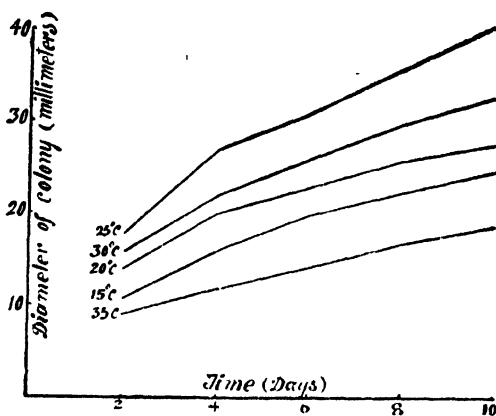
Fig. 8. Temperature relationship of *C. sesami* on Hopkins' agar
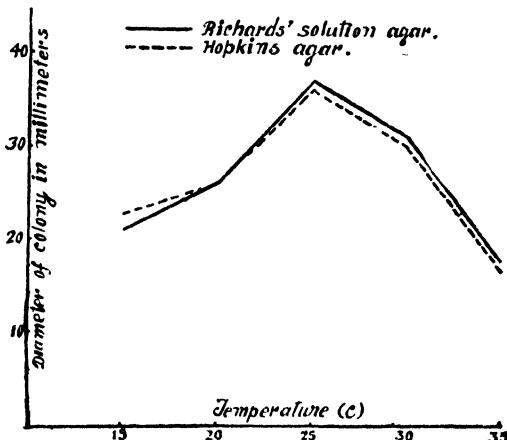
Fig. 9. Eight days' growth of *C. sesami* on Richards' solution agar and Hopkins' agar at various temperatures

It will be seen from these figures that the fungus grows well between 20° C. and 30° C. and that temperatures above and below are detrimental to the growth of the fungus. It will also be noticed that the optimum temperature for growth lies very probably between 25° C. and 30° C. At higher temperatures an abnormal type of growth takes place and the colony becomes pale and forms a lot of felty aerial mycelium.

(vi) *Concentrations*

The fungus was grown on 10 N, 5 N, 2 N, N, N/2, N/5, N/10, N/20, N/50 and N/100 concentrations of Coons' solution in flasks of uniform capacity containing 100 c.c. of the medium. Inoculated flasks were kept at 25° C. and the mycelium was filtered after 31 days and

the dry weight of the fungus determined. The experiment was run in triplicate and the data obtained, which is the average of the three, are shown in Table V.

TABLE V

Average dry weight of the mycelium in gms. of C. sesami *at various concentrations of Coons' synthetic solution*

Concentrations	Average dry weight of the mycelium in gms.
10 N	0·5672
5 N	0·2706
2 N	0·1461
N	0·0678
N/2	0·0281
N/5	0·0102
N/10	0·0017
N/20	0·0013
N/50	0·0009
N/100	0·0003

From the data presented in Table V it will appear that the dry weight of the mycelium increases in direct proportion to the increase in concentration of the medium and decreases with the decrease in the concentration of the normal solution. The results obtained agree with those of Moore (1924) and Brown (1925).

(vii) *Importance of the different constituents of Richards' solution*

The importance of the different constitutents of Richards' solution on the growth of the fungus was determined by eliminating from the culture solution each salt one by one. The data so obtained are presented in Table VI.

TABLE VI

Average dry weight of mycelium of C. sesami *grown on Richards' solution and those lacking in one of its constituents at* 25° C. *after* 30 *days*

Media	Dry weight of the mycelium in gms.
Normal	0·314
No FeCl₃	0·261
No KH₂PO₄	0·163
No MgSO₄	0·132
No KNO₃	0·034
No sugar	..

It will appear from Table VI that sugar is the most important constituent of the medium. Without sugar there was practically no growth. Next to sugar KNO_3 seems to be most essential. The absence

of ferric chloride was much less felt than either $MgSO_4$ or KH_2PO_4. In normal medium the fungus showed the best growth.

(viii) *Zonation*

Concentric zones were noticed in all the plates, faintly in some and distinctly in others. Zones have been noticed in the growths of other fungi and the formation of these has been attributed to various causal agencies, ' to light relation, to nutrients, to agencies other than light probably food, to resting periods and to mycelial crowding ' (Stevens and Hall, 1909). Brown (1925) has observed in the growth of certain strains of *Fusarium* definite series of rings corresponding to the alteration of day and night. Bisby (1925) in his review of the literature on the subject has stated that different fungi gave variable results.

In this fungus the number of zones differed in different media. They were most sharply defined on oat meal agar, Richards' solution agar and Coons' agar. On Hopkins' and Dox's agar no zonation could be noticed.

With regard to this fungus it was also found that the formation of the zones is due to alternation of light and darkness. The zones were absent in cultures kept in continuous darkness and in continuous light but quite distinct in those kept outside exposed to light during the day and to darkness during the night.

Temperature has also been noticed to play an equally important part in zone formation. This fact has also been observed by Christensen (1926) in the case of *Helminthosporium sativum* and by Mitra and Mehta (1934) in the case of *H. nodulosum.* Bisby (1925) obtained similar results from *Fusarium discolour sulphureum* and Coons and Larmer (1930) from *Cercospora beticola.*

Zonation can be induced by fluctuating of temperature within a certain range. The data of zonation in relation to temperature are given in Table VII.

TABLE VII

Relation of temperature to zonation in C. sesami
in darkness in Dox's agar

Temperature °C.	Zonation	Remarks
10	Nil	Constant
20	,,	,,
30	,,	,,
35	,,	,,
20–30	Traces	Alternating
20–25–30	Good	,,
35–38	Nil	,

In *C. sesami* at a constant temperature zones either failed to appear or were only very faintly visible. The alternation of temperature within 20°–30° C. resulted in the appearance of zones, but above or below this range zonation could not be induced.

B. Microscopic characters

(i) *Character of the mycelium*

The mycelium when young is septate at long intervals and is sub-hyaline ; with age it becomes light brown to brown and septa appear at short intervals. In older cultures it becomes dark brown, highly tortuous and toruloid.

(ii) *Factors affecting sporulation*

(a) *Light.*—Continuous light or darkness has been found to inhibit sporulation. Cultures kept in alternate light and darkness (day and night) have been found to sporulate earlier and more copiously than those kept in continuous light or darkness. It was further observed that when the fungus was grown in petri dishes in darkness and after a few days the growth was exposed to light the formation of spores was stimulated.

(b) *Humidity.*—Conditions of high humidity also seemed to favour sporulation. Plates with growing cultures of the fungus held upside down over water surface and various percentages of sulphuric acid showed differences in sporulation. Conditions of high humidity from 80 to 100 per cent. favoured sporulation while at a lower humidity its formation was entirely suppressed.

Sometimes sporulation has been found to be copious in cultures saturated with water. When bits of agar containing mycelium of the fungus were placed in watch glasses and saturated with distilled water copious sporulation took place.

(c) *Temperature.*—Change of temperature was found to have a stimulating influence on sporulation. Sporulation was accelerated by growing the fungus at low temperatures ($10°$–$15°$ C.) and after about 10 days' growth exposing the culture to higher temperatures ($30°$ C.).

(d) *Media.*—Other factors being the same sporulation varied on different media. In general sporulation was more liberal when the fungus was grown on meal agars than on synthetic media. The most copious spore production, however, took place when the fungus was grown on sterilized *Sesamum* stems in tubes.

TABLE VIII

Sporulation of C. sesami on different media

Sterilized *Sesamum* stems	.. Very good
Oat meal agar	.. Good
Maize meal agar	.. Good
Hopkins' agar	.. Moderate
Dox's agar	.. Moderate
Browns' synthetic agar	.. Poor

(e) *Wounding.*—In certain instances sporulation is accelerated in a culture by local wounding. Petri dish cultures which were wounded here and there were found to sporulate fairly strongly along the lines of wounding.

(iii) *Size and septation of conidia*

The size and septation of conidia have been found to vary under different environmental factors, the most important being temperature, relative humidity and media.

(a) *Temperature.*—An important factor in affecting the size and septation of conidia is temperature. The shape of the conidia is, however, not much affected. The spore size and septation at different temperatures are given in Table IX.

TABLE IX

Variation in size and septation of conidia of C. sesami
on Hopkins' agar at various temperatures

Temperature	Length		Width		Septation			
	Range μ	Mean μ	S.D. μ	Range μ	Mean μ	Range	Mean	Mode
C 20	64–015	87·64 ±0·29	7·5 ±0·207	2·5–4	3·5	6–10	8	8
25	86–130	109·50 ±0·38	9·9 ±0·273	2·5–4	3·4	7–10	9	8
30	77–123	99·60 ±0·39	10·1 ±0·279	2·0–3·5	3·4	7–10	8	7
35	64- 96	79·50 ±0·38	8·5 ±0·275	1·5–3·0	2·5	5- 8	6	5

From Table IX it will be seen that the mean length of the conidia is the greatest at 25° C. and the least at 35° C.; conidia formed at 20° C. and 30° C. have their mean length less than those formed at 25° C. but more than those formed at 35° C. The mean width decreases with the rise in temperature and the mean septation is the greatest at 25° C., same at 20° C. and 30° C. and the least at 35° C.

(b) *Relative humidity.*—Welles (1925), Klotz (1923) and Sundara-raman and Ramakrishnan (1928) and Ramakrishnan (1931) found on different species of *Cercospora* that remarkable increase in size and septation of conidia takes place at high humidities. An experiment was carried out by incubating infected leaves at relative humidities of 47, 70·4 and 100 per cent. and after forty hours a count of 300 conidia were made at random. The results are recorded in Table X.

TABLE X

*Effect of different relative humidity on size and septation
of conidia of* C. sesami

Relative Humidity	Length			Width		Septation		
	Range μ	Mean μ	S.D. μ	Range μ	Mean μ	Range	Mean	Mode
47·0	86–125	102·93 ±0·33	8·7 ±0·24	3–4·0	3·5	7–10	8·5	8
70·4	96–169	128·21 ±0·59	15·3 ±0·42	3–3·5	3·2	7–12	10·5	10
100·0	86–245	157·86 ±0·97	23·5 ±0·69	3–3 5	3·2	7–17	13·0	11

It will be seen from Table X that the average length of conidia increased and the average width decreased with the rise in relative humidity. The number of septa also increased with the increase in the relative humidity.

(*c*) *Media.*—The size and septation of conidia depend on the nature of the medium on which the fungus is cultivated. Conidia measurements were made from fifteen days' old cultures grown on Dox agar, sterilized *Sesamum* stems and oat meal agar and are shown in Table XI.

TABLE XI

Variation in size and septation of conidia of C. sesami
on different media

Media	Length			Width		Septation		
	Range μ	Mean μ	S.D. μ	Range μ	Mean μ	Range	Mean	Mode
Sesamum stem	87–135	110·88 ±0·40	10·3 ±0·28	3 4	3·4	7· 10	9	9
Oat agar ..	80–127	105·0 ±0·38	10·0 ±0·27	2·5–3·5	3·0	6– 8	7	7
Dox agar ..	80–120	101·76 ±0·42	10·9 ±0·42	2·5–3·	3·0	6– 8	7	6

It will be seen from Table XI that under identical conditions the average length of the conidia is the greatest on *Sesamum* stems and the least on Dox agar ; the average width was same in Dox and oat meal agars and slightly more on *Sesamum* stems. Number of septa was the greatest on *Sesamum* stems and same on Dox and oat meal agars.

(iv) *Secondary spores*

The formation of secondary conidia from primary conidia often takes place both in nature as well as in culture. In some cases the first formed conidium produces a second one at its tip, while in others lateral conidia are formed from the sides of the basal cells of the larger conidia (Fig. 5). The secondary conidia so formed are small and usually non-septate, rarely one septate.

(v) *Spore germination*

Under the most favourable conditions conidia may germinate within 4 to 6 hours but they usually require longer and some do not germinate for days, although to all appearances conditions are favourable.

The conidia readily germinate in tap watet within 4–6 hours. Germ tubes are given off (Fig. 6) from both ends of spore as well as from sides near the septa. At times two germ tubes originate from

a single cell of a conidium. Conidiophores and even bits of them also readily germinate in tap water (Fig. 3).

The time required for the germination of spores varies with the culture media used, the age of the spores, temperature, hydrogen-ion concentration and humidity.

(a) *Sugar.*—Sugar solutions have been found to exert a favourable influence on the germination of spores. Spores which germinated slowly in distilled water and tap water did so more quickly when placed in sugar solutions ; a higher percentage of spores germinated and the germ tubes also attained greater length. The results obtained are given in Table XII.

TABLE XII

Effect of sugar on the germination of spores of C. sesami

Media	After ten hours	
	% of germination	Av. length of germ tube s(μ)
Distilled water	25·0	12·6
Tap water ..	32·5	20·9 .
5% glucose solution ..	86·9	45·?
5% succrose solution	88·4	44·0

(b) *Temperature.*—Spores were taken from a 15 days' old culture on *Sesamum* stems and spore suspensions made in sterilized distilled water. A drop of the spore suspension was placed in the centre of a cover slip and inverted over Van Tiegham rings containing a few drops of distilled water. These were then exposed for 12 hours at 20° C., 25° C., 30° C. and 35° C. temperatures. Percentage germination of spores and the average length of the germ tubes for each temperature were determined and the results are recorded in Table XIII.

TABLE XIII

Germination of conidia of C. sesami *at various temperatures*

Temperature ° C.	% of germination	Av. length of germ tubes (μ)
20	72	37·5
25	80	62
30	61	51·0
35	32	19·1

It will be found from Table XIII that the percentage of germination as well as the average length of germ tubes is the greatest at 25° C.

(c) *Humidity.*—The effect of various percentages of relative humidities on the germination of the spores of *C. sesami* was determined according to the method of Lesage (1895). The principle involved is that the saturation of the air above a given solution of sodium chloride varies inversely with the concentration of the salt dissolved therein and the humidity is said to remain constant though the

temperature may vary.   Moisture free sodium chloride was taken and
solutions containing 4·5 to 25·0 gms. per 100 c.c. of water were
prepared and 25 c.c. of the solution was put in dishes of 6 cm.
×3·5 cm.   A drop of distilled water containing a spore suspension
was spread on the underside of each of the lids and dried *in vacuo*
over calcium chloride.   The lids were then placed over the dishes
and sealed with a 'mixture of paraffin and vasaline.   All the dishes
were placed in an incubator running at a constant temperature of
25° C.   Spores were examined after 6 hours and 24 hours and the
results obtained are given in Table XIV.

TABLE XIV

Germination of spores of C. sesami *at various relative humidities*

Gms. NaCl	Relative Humidity	6 hours		24 hours	
		% germina-tion	Av. length of germ tubes (μ)	% germina-tion	Av. length of germ tubes (μ)
0·0	100	45·5	24·2	89·6	69·5
4·5	97·3	29·6	15·8	66·0	42·2
7·0	95·7	16·6	11·5	41·2	31·4
10·0	93·4	8·4	8·0	18·1	19·9
12·0	92·7	5·9	4·7	12·4	11·5
15·0	91·0	..	..	..	..
16·0	90·0	..	..	..	..

It will be evident from Table XIV that the best germination was
obtained at 100 per cent. relative humidity and it gradually decreased
upto 91 per cent. when there was no germination.

   (*d*) *Hydrogen-ion concentration.*—Spore germination is remarkably
affected by hydrogen-ion concentration of the solution.   Spore suspen-
sions were made in solutions of known pH values and a drop mounted
on a cover slip and inverted over Van Tiegham rings containing
a few drops of the solution of the same pH values as the hanging drop.
Adjusted modified Richards' solution of Karrer and Webb (1920)
was used and the solutions of the following pH value were prepared :
2·1, 2·5, 2·7, 2·8, 3·3, 4·9, 5·9, 6·9, 7·3, 8·0 and 9·1.   All the
slides were incubated at 25° C. for 10 hours.   The percentage of
germination and the average length of the germ tubes were determined
for each pH value.   The results are recorded in Table XV.

TABLE XV

Germinatnation of spores of C. sesami *at different*
hydrogen-ion concentrations

pH values	2·1–2·8	3·3	4·9	5·9	6·9	7·3	8·0	9·1
Percentage germination ..	..	51	70·0	97·0	24·5	11·5	6·0	..
Av. length of germ tubes (μ)	..	14	16·4	42·7	12·3	3·6	3·0	..

It will be evident from Table XV that the spores can tolerate a wide range of hydrogen-ion concentration from 3·3 to 8·0. The minimum hydrogen-ion concentration for germination lies between pH 2·8 and 3·3 and the maximum lies between 8·0 and 9·1 and the optimum is between 4·9 and 5·9.

VI.   HYDROGEN-ION CONCENTRATION

In order to determine the hydrogen-ion concentration relationship of the growth of *C. sesami* Richards' solution as modified by Karrer and Webb (1920) was used and his method was followed. Thirty c.c. of the solution together with the required amount of N/5 acid and N/5 alkali and distilled water to make 50 c.c. was put in each flask and hydrogen-ion concentration was determined according to the colorimetric method of Clark and Lubs (1917). Four flasks were prepared for each pH value ; one of these was put as control while the other three were inoculated. All the flasks were incubated for 56 days at 25° C. after which the dry weight of the mycelium of the fungus was determined. The hydrogen-ion concentrations of the controls and filtrate were also determined to see whether changes were brought about as a result of the metabolic activities of the fungus. The data obtained are given in Table XVI and represent the average dry weight of the mycelium from three flasks in each case.

TABLE XVI

Growth of C. sesami *at different pH and the changes in reaction induced*

Hydrogen-ion concentration			Average dry weight of the mycelium
Initial	Control after 56 days	Inoculated after 56 days	
2·1–2·7	2·1–2·7	2·1–2·7	No growth
3·3	3·3	2·6	0·1265
4·6	4·2	2·6	0·1621
5·5	4·9	2·6	0·1625
6·5	5·7	2·6	0·194
7·1	6·4	2·6	0·1901
8·1	6·4	6·6	0·0260
8·5	6·9	6·9	No growth

It will be noticed from Table XVI that the growth occurs over a range of pH varying from 3·3 to 8·1. Maximum growth occurs at 6·5 ; on the acid side no growth takes place beyond 3·3 and on the alkaline side beyond 8·1. The fungus during its growth produced marked changes in the reaction of the medium. It is seen from the table that there has been very little change in the pH values of the controls on the acid side of neutrality. while in those on the alkaline side there is remarkable shift in the pH values. The pH 2·1 to 2·7 remained constant after 56 days while pH 7·1, 8·1 and 8·5 shifted to

pH 6·4, 6·4 and 6·9 respectively. The pH 3·3, 4·6, 5·5, 6·5 and 7·1 of the inoculated flasks all shifted to a consatnt of 2·6 which therefore represents the acidity of the medium oh which the fungus grew well.

VII. SUMMARY

Cercospora sesami Zimm. is parasitic on leaves, petioles, stems and pods of *Sesamum indicum*. Symptoms of the disease have been described and the morphology of the parasite given in the text.

Inoculation experiments show that all the parts of *Sesamum indicum* are susceptible to the attack.

A study of the fungus was made on a large number of artificial media. Its growth is better on thickly poured plates than on thinly poured ones, more in alternate light and darkness than in complete darkness or continuous light.

The fungus can tolerate a wide range of relative humidity from 50 to 100 per cent., the optimum humidity for growth being 92 per cent.

The temperature relationship has been studied on Hopkins' and Richards' solution agar and the optimum has been found to lie between 25°—30° C.

On Coons' solution it has been found that the dry weight of the mycelium decreases with the decrease in concentration of the medium from the normal and increases with the increase in concentration above the normal.

Sugar is the most important constituent of Richards' solution influencing the growth of the fungus ; KNO_3, $MgSO_4$, KH_2PO_4 and $FeCl_3$ are next in importance in the order mentioned.

Media, alternate light and darkness and fluctuating temperatures are the most important factors influencing zonation in artificial culture.

Light, humidity, temperature, media and wounding have been found to influence sporulation in culture ; the details are given in the text.

The size and septation of the spores are influenced by temperature, humidity and media.

The effect of sugar, temperature, humidity and hydrogen-ion concentration on spore germination was studied and the details are described in the text.

The optimum hydrogen-ion concentration for the growth of the fungus is at 6·5. There is no growth at 2·1 to 2·7 and 8·5.

FIG 1. *Cercospora sesami* on *Sesamum indicum.* Symptoms of the disease

S. CHOWDHURY—PHYSIOLOGY OF *CERCOSPORA SESAMI* ZIMM.

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THE ENZYMES OF TWO WATER MOLDS

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INTRODUCTION

THE enzymic reactions are absolutely necessary for any process associated with the cellular activity of a living organism. So far very little work seems to have been done with regard to enzymes of the members of the family Saprolegniaceæ. Of the various genera, *Achlya* sp. and *Saprolegnia Tokugawana* were studied by Emoto (1923) ; *Achlya bisexualis* and *Saprolegnia ferax* by Wolf (1937) and *Saprolegnia delica* by Saksena and Bhargava (1941). Very recently Bhargava (1943) studied the more important intra- and extra-cellular enzymes of *Achlya* sp., *Brevilegnia gracilis*, *Isoachlya* sp., *Saprolegnia delica* and *S. monoica* both qualitatively and quantitatively.

The present work deals with the estimation of more important extra- and intra-cellular enzymes present in *Achlya dubia* Coker and *Thraustotheca clavata* (deBary) Humph.

MATERIAL AND METHODS

Achlya dubia Coker was isolated from a local sample of water using ants as baits. Single spore cultures were prepared by the usual method. The culture of *Thraustotheca clavata* (deBary) Humph. was obtained from Centraal Bureau voor Schimmelcultures, Baarn, Holland. Throughout the investigation, the methods adopted by Bose and Sarkar (1937) and Bhargava (1943) were closely followed. For the details of experiments, the reader is referred to his paper (Bhargava, 1943). Since the fungi remain sterile in the medium used, determination of enzymes was made only at one stage, *i.e.*, of vegetative mycelium.

EXPERIMENTAL

Carbohydrases.—The presence of carbohydrases, *viz.*, amylase invertase, maltase, emulsin, cellulase and hemicellulase in the mycelium of the two organisms was tested qualitatively by their ability to reduce the solutions of the corresponding carbohydrates, such as, soluble starch, cane sugar, maltose and amygdalin, etc. To determine the amount of enzymic activity quantitatively, a reaction mixture was prepared together with a suitable buffer solution and the amount of reduced sugar was estimated by Shaffer and Hartman's method (1921). The mean results are given in Table I.

TABLE 1

Amount of reducing sugar (in mgs.) formed in 10 *c.c. of the total digested volume out of* 37 *c. c.*
containing 0·1 *gm. of fungus meal*
or 2 *c.c. of extra-cellular enzymic solution*

Enzyme	*Achlya dubia*		*Thraustotheca clavata*	
	Intra-cellular enzymic activity	Extra-cellular enzymic acitvity	Intra-cellular enzymic activity	Extra-cellular enzymic activity
Amylase ..	8·45	1·95	11·45	4·5
Invertase ..	5·25	0	8·65	0
Maltase ..	10·2	4·0	10·45	1·55
Emulsin ..	36·75	2·75	32·7	2·35
Cellulase ..	9·55	9·05	10·5	9·05
Hemicellulase ..	8·6	13·15	5·5	8·1

The results obtained in Table 1 indicate that generally the intra-cellular activity is much greater than the extra-cellular except in the case of hemicellulase. Invertase is absent as an extra-cellular enzyme in both the species.

Proteolytic Enzyme.—A suitable reaction mixture prepared with peptone, cirtate buffer and fungus meal or extra-cellular enzymate solution, was incubated in flasks for 24 hours at 37° C. and then 2 c.c. of the reaction mixture was titrated with 0·01 alcoholic KOH solution. The results obtained are gievn in Table II.

TABLE II

Amount of KOH solution (in c.c.) for 2 *c.c. of the total digested volume of* 12 *c.c. containing* 0·1 *gm. fungus meal or* 2 *c.c. of the extra-cellular enzyme solution*

Fungus	Intra-cellular enzymic activity		Extra-cellular enzymic activity	
	Control (inactivated)	Active enzyme	Control (inactivated)	Active enzyme
Achlya dubia	14·95	14·0	16·75	16·15
Thraustotheca clavata ..	14·95	14·5	16·75	14·5

The results obtained in Table II show the presence of proteolytic enzymic activity.

Lipase.—The presence of lipolytic enzyme was estimated by the method of Kanitz (1925). The reaction mixtures were titrated with N/25 KOH solution. The results are given in Table III.

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TABLE III

Amount of 0·04 *N KOH* (*in c.c.*) *for the whole digested volume containing* 0·1 *gm. of fungus meal or 2 c.c. of extra-cellular enzyme solution*

Species	Intra-cellular enzymic activity		Extra-cellular enzymic activity	
	Control (inactivated)	Active enzyme	Control (inactivated)	Active enzyme
Achlya dubia	6·1	5·8	3·85	3·85
Thraustotheca clavata ..	6·2	5·9	2·95	2·95

From Table III it is seen that lipase is absent as an extra-cellular enzyme in both the species. There is, however, a slight intra-cellular activity.

Butyrase.—The flasks containing the suitable reaction mixtures were incubated at 40° C. for 5 days and were then titrated with N/50 KOH solution. The results are tabulated in Table IV.

TABLE IV

*Amount of N/*50 *KOH* (*in c.c.*) *solution required to neutralise the acid given out in hydrolisation in* 10 *c.c. of the reaction mixture*

Species	Intra-cellular enzymic activity		Extra-cellular enzymic activity	
	Control (inactivated)	Active enzyme	Control (inactivated)	Active enzyme
Achlya dubia	7·65	5·2	5·2	4·8
Thraustotheca clavata ..	7·65	5·0	5·2	4·7

The above results show that butyrase is present both as an extra- and intra-cellular enzyme.

Catalase.—The suitable reaction mixture was incubated at room temperature for two hours, after which it was titrated with 0·1 N $KMnO_4$ in presence of sulphuric acid. The results obtained are given in Table V.

TABLE V

Amount of 0·1 *N KMnO$_4$* (*in c.c.*) *required for 5 c.c. of hydrogen peroxide solution containing* 0·1 *gm. fungus meal or 2 c.c. extra-cellular enzyme solution*

Species	Control (inactivated)	Intra-cellular enzymic activity	Extra-cellular enzymic activity
Achlya dubia	2·0	1·0	2·0
Thraustotheca cavaata ..	2·0	1·5	2 0

It is seen that the enzyme shows no extra-cellular activity, though it is present as an intra-cellular enzyme.

Laccase, Tyrosinase, Rennetase and Oxidase.—Various tests were made to test the presence of laccase, tyrosinase, rennetase and oxidase. The results obtained indicate that the above mentioned enzymes are altogether absent in the two species studied by the authors.

DISCUSSION

The function of carbohydrases is to convert more complex carbohydrates into such simpler compounds as can be used directly by the organism. The presence of a particular enzyme in an organism indicates that the organism is able to utilise the corresponding carbohydrate. From the results obtained in Table I, it is evident that the fungi under investigation, *viz., Achlya dubia* and *Thraustotheca clavata* are able to secrete all the carbohydrases tested, except invertase. This shows that sucrose will not be assimilated by them. The results obtained in the present case are in entire agreement with those obtained by Bhargava (1943) for some of the water molds used by him. The present organisms too, like other watermolds, differ from *Brevilegnia gracilis*, a parasite, but a member of the family Saprolegniaceæ.

Like the results obtained by Emoto (1923) and Bhargava (1943), proteolytic activity, though feeble, is present in *Achlya dubia* and *Thraustotheca clavata.*

As regards lipase, Emoto (1923) reported its absence in *Achlya* sp. and *Saprolegnia Tokugawana* ; Wolf (1937) found it to be present in *Achlya bisexualis* and *Saprolegnia ferax* : Saksena and Bhargava (1941) pointed out that it was not secreted by *Saprolegnia delica* and Bhargava (1943) also obtained similar results with other fungi. In the present instance also it is not secreted by the fungi investigated.

Butyrase is present both as an intra- and extra-cellular enzyme. The results obtained by Bhargava (1943) show that it was present as intra-cellular enzyme only in the case of water molds studied by him.

Catalase has been found to be present as an endoenzyme only.

The present authors also, like Bhargava (1943, p. 97), are inclined to the view that the activation of the atmospheric oxygen into a more chemically reactive state is not required by the water molds. Therefore oxidase, laccase, tyrosinase and rennatase which are oxidising enzymes, are not produced in the species under investigation.

SUMMARY

Some of the more important enzymes of *Achlya dubia* Coker and *Thraustotheca clavata* (deBary) Humph. have been estimated.

Among the carbohydrases amylase, maltase, emulsin, cellulase and hemicellulase were present both as extra-cellular and intra-cellular enzyme. Invertase was present as intra-cellular enzyme only. In all cases except the hemicellulase the amount of intra-cellular enzyme was greater than the corresponding extra-cellular one.

Proteolytic enzyme was present in small quantities. Lipase and catalase showed only a slight intracellular activity, while butyrase was present both as extra- and intra-cellular enzymes.

Laccase, tyrosinase, rennetase and oxidase were found to be absent.

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THE VEGETATION OF THE RAJGHAT RAVINES

By R. Misra

Benares Hindu University

I. Situation. Topography and Development of the Ravines

A LARGE number of ravines bearing forest growth dissect the sides of the plateau of Rajghat as it stands to the north-east of Benares town separated from it by a depression carrying the Grand Trunk Road and Kashi station of the East Indian Railway situated on the ridge. The plateau itself is a rectangular area about ¾ mile long and ¼ mile broad with the Ganges scoring on the south and the river Barna circumscribing it on the north and the east where it meets the former river (Fig. 1). It is about 255 feet above the sea and the rivers run 55 feet below during the dry season. The exposed banks of the rivers are then cultivated.

The ravines are formed on account of gully erosion. The monsoon bringing the rain releases it generally in a torrential downpour during the rainy season. The water collecting and running on the periodically rain battered ground of the plateau scores out small gullies. As the various ramifications of these unite and the intensity of soil erosion increases with the increasing volume of running water the ravines are dug deeper and wider at lower levels when approaching the rivers. Occasional landslides cut the sides precipitously giving them the appearance of deep gorges. The top of the plateau slopes gradually from the south to the north and hence the ravines are longer towards the Barna than those running into the Ganges.

II. History and Biotic Factors

Although the development of the ravines is so rapidly ruinous to the plateau yet it has a marvellously long history of existence though undoubtedly getting spatially narrowed on the north and the south. The area was once thickly inhabited as is clear from the traces of old buildings, the Rajghat Fort and from the more recent excavations conducted by the department of Archæology. It is now the property of the Rishi Valley Trust who have built on the site a residential school and college. The buildings including the old temple of Adikeshwar extend up to the confluence of the rivers.

The stability of the plateau is chiefly dependent on the interesting vegetation of the ravines which has appreciably slowed down the progress of erosion if it has not already arrested it at certain places. Wherever the growth of this vegetation has been interfered with it
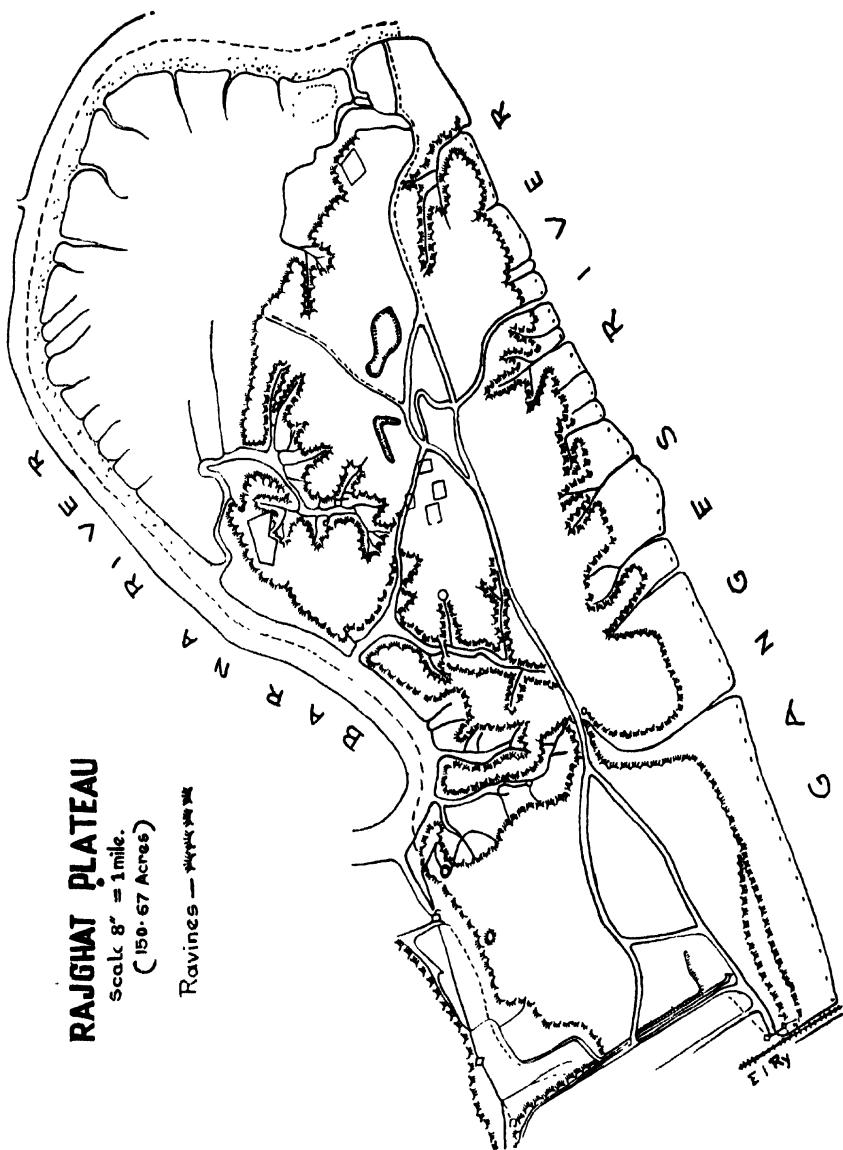
RAJGHAT PLATEAU
Scale 8" = 1 mile.
(150·67 Acres)

Ravines —

BARNA RIVER

RIVER

GANGES RIVER

E I Ry

FIG. 1

has led to ruin and misery. The clearing of the vegetation for the archæological excavations on the south-west corner of the plateau and the college buildings on the north-eastern ridge has already made their position precarious. Further, the ravines contain abundant remnants of buildings including a huge stone gate of the old fort which has collapsed on the Barna side. These point to the havoc wrought by erosion due to the destruction of the vegetation subject to periodic pressure of human population on the historical plateau.

Clearing of the vegetation especially about a ridge or a rudimentary ravine for building purposes is therefore a process ruinous to the vegetation and the plateau. Felling the trees for timber and fuel and lopping are common practice and continuous grazing of cattle, sheep and goat limit the growth of the forest in the ravines. Scraping of the ground vegetation which may at times cover parts of the plateau exposes the loosened soil for rapid erosion. Most of the herbs including the taller rainy season annuals dry up in the cold season when they are collected for fuel and not allowed to decay on the soil which might improve its fertility.

III. Climate*

The periodic climate of Benares is typical of the Upper Gangetic Plain with an annual average rainfall of 40 inches. The rainy season extends from about the middle of June to September when nearly 36 inches of the fall are obtained ; July and August being the wettest months. The average number of rainy days in the season is 20 per month, and the mean relative humidity is about 88%. The mean maximum temperature during the period is about 90·5° F. and the corresponding minimum is 78° F. This is the most favourable season for plant growth as it follows the dry hot summer.

The bright month of October is rainless and calm. The mean maximum temperature is 94·7° F. but the mean minimum is 65·6° F. on account of the cooler nights. This is followed by the cold season of 4 months when the days are cool and bright with a breeze from the west. The nights are cold and humid. There may be a little rainfall late in the season but it seldom exceeds 2 inches in any month. The mean maximum and minimum temperatures for the season are 80·8° and 52·8° F. and the mean relative humidity is 79·9%.

The month of March is a mild warm period of transition between the cold season and the following hot season ; the latter extends from April to the middle of June. The mean maximum temperature during the hot season is about 105° F. but the absolute maximum temperature may go upto 115° F. in May. Dry hot wind known as " loo " blows strongly from the west during the day time. It desiccates plant tissues very often killing them. But the narrower ravines are not· much exposed and as the wind sweeps over the rivers before turning into them the protected vegetation does not suffer much on this

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The figures given in this section are based on records for 1942-43 (Misra. 1944).

account. The nights are not so hot and the mean minimum tempe-
rature for the season is 76·6° F. The mean relative humidity for the
period is about 48%. The weather becomes stormy at times with
occasional drizzles following dust. However, erosion of soil by wind
is moderate.

The ravines in the south to the Ganges side receive more of
sun than those in the north towards the Barna. This factor seems
to be responsible for differences in the floristic details of the plant
communities present in the two systems.

IV. SOIL

The plateau consists of a fairly uniform deposit of an old alluvium
on a bed of ' kankar ' ; the latter is found locally exposed on the
bank of the Barna. The soil is light being moderately porous and
gray and brown coloured with small rounded grits and calcareous
nodules. It is alkaline with pH values ranging between 7·90 and
8·34 and it gives a strong positive reaction for nitrates when tested
with a 0·02% solution of di-phenyl-amine in concentrated sulphuric
acid (Misra, 1944). The ravines at their lower approaches to the
river get covered with newer sandy deposits every year on inundation
when it is in spate during the latter part of the rainy season. The
coarser soil is pale coloured and generally richer in carbonates.

V. VEGETATION

1. *General characters of the vegetation*

The pioneer species which consolidates the soil against erosion
in the area is *Capparis sepiaria*. On account of the thorns on the plant
it also affords protection to the vegetation growing under it against
grazing. As a result *Diospyros cordifolia* followed by a number of
deciduous trees rapidly grow up to form a closed forest. Most of
these and especially the two species named above are capable of
regenerating from buds developing on their roots as they get exposed
on erosion of the soil. These can therefore establish along with some
annuals on the precipice also. Thus the ravines get completely
covered with a thick mantle of forest which becomes quite impenetrable
by the middle of the rainy season every year so effectively checking
soil erosion indeed, at a time when its absence might be disastrous
to the plateau.

With the dying of the annuals, slower growth of the perennials
and indiscriminate grazing and removal of wood for fire in the
following cold season the forest becomes thinner. The uppermost
storey consisting of the deciduous trees opens up still further by the
fall of the leaves in the beginning of the hot season. The forests of
the ravines become poorer with the march of the dry seasons since
the intensity of the biotic factors increases simultaneously with a
gradual decline in the growth of the vegetation. Continued grazing
and scraping of the ground vegetation prepare the plateau for a
fresh assault by the monsoon erosion which has been shown to be
severe in the earlier part of the rainy season.

2. *Plant communities*

The following plant communities have been recognised in this study. The species marked with an asterisk are either exclusively confined or more abundant in the sunny Ganges ravines. The notations used are:—*d*–dominant, *cd*–codominant, *a*–abundant, *f*–frequent, *o*–occasional, *r*–rare : *v* and *l* used as prefix signify 'very' and 'local' :--

1. *Holoptelia— Albizia—Cordia* association

Holoptelia integrifolia, Planch.	d	*Feronia elephantum*, Correa.	*l*
Albizia lebbek, Benth	cd	*Tamarindus indica*, L.	r
Cordia myxa, L.	cd	*Ficus religiosa*, L.	r
Pongamia glabra, Vent.	o-f	*Ehretia acuminata*, Br.	*vr*
Melia Azadirachta, L.	o	*Mitragyna parvifolia*, Korth.	*lr*
Acacia leucophlaea, Willd.	o		

2. Consociations of *Holoptelia integrifolia, Albizia lebbek* and *Cordia myxa*

3. Societies of *Pongamia glabra* and *Acacia leucophlæa*

4. *Capparis—Diospyros* associes

Capparis sepiaria, L.	d	*Lantana indica*, Roxb.	f
Diospyros cordifolia, Roxb	cd	*Cocculus villosus*, DC.	r
Capparis horrida, L.	o	*Coccinia indica*, W. and A.	o
Clerodendron phlomidis, L.	o	*Quamoclit pinnata*, Boj.	o
Abrus precatorius, L.	o	*Rhynchosia minima*, DC.	o
Abutilon indicum, G. Don.	o	*Cardiospermum halicacabum*, L	*l*
A. graveolens, W. and A.	o	*Melothria heterophylla*. Cogn.	

The climbers become more abundant during the rainy season.

5. Consocies of *Capparis sepiaria* and *Diospyros cordifolia*

6. Associes of *Ficus glomerata—Pongamia glabra*

Ficus glomerata, Roxb.	d	*Ficus religiosa*, L.	o
Pongamia glabra, Vent.	cd	*Dalbergia sissoo*, Roxb.	*r*

7. *Saccharum* consocies

Saccharum munja, Roxb.	d	*Desmostachya bipinnata*, Stapf	f-a
S. Ravennae, L.	f	*Indigofera tinctoria*, L.	o
Alhagi camelorum, Fisch.	a		

8. A scrub colony

Acacia arabica, Willd.	f	*Phoenix sylvestris*, Roxb.	f
Zizyphus jujuba, Lamk.	f	*Streblus asper*, Lour.	o

9. Colonies and societies of herbs

   (*a*) On precipice :

Aerua scandens, Wall.	*Linaria ramosissima*, Wall.
Aristida adscensionis, L.	*Lindenbergia urticaefolia*, Lehm.
Cenchrus ciliaris, L.	*Peristrophe bicalyculata*, Nees.
Chloris virgata, Sw.	*Pulicaria crispa*, Benth.
Digitaria sanguinalis, Scop.	

(b) On moderately eroded land :

Abutilon indicum, G. Don.
Aerua scandens, Wall.
Blepharis boerhaaviaefolia. Pers.
B. molluginifolia, Pers.
Desmostachya bipinnata. Stapf.
Dichanthium annulatum, Stapf.

Indigofera tinctoria, L.
Nepeta ruderalis, Ham.
Pulicaria crispa, Benth.
Pupalea lappacea, Moq.
Saccharum munja, Roxb.

(c) On open grounds :

Achyranthes aspera, L.
Blumea spp.
Boerhaavia diffusa. L.
†*Cassia occidentalis*, L.
†*C. tora*, L.
†*Crotalaria medicaginea*, Lamk.
Cynodon dactylon. Pers
Cyperus rotundus. L.
Dichanthium annulatum, Stapf.
Digera arvensis, Forsk.
†*Digitaria sanguinalis*, Scop.

Euphorbia hirta, L.
E prostrata, Ait.
E. thymifolia, L.
Indigofera ennaephylla, L.
Jatropha gossypifolia, L.
Justicia diffusa, Willd.
Ocimum canum, Sims.
Sida rhombifolia, L.
†*Urochloa reptans*, Stapf.
Vernonia cineria, Less.

(d) On shaded grounds :

Acalypha ciliata, Forsk.
A. indica, L.
Achyranthes aspera, L.
Anisomeles ovata, R.
Barleria prionitis, L.
Biophytum sensitivum, D. C.
Boerhaavia repanda, Willd.
†*Commelina benghalensis*, L.
†*Corchorus acutangularis*, Lam.
Desmodium gangeticum, DC.
Eleusine aegyptica, Desf.
Eragrostis tenella, Stapf.
Hyptis sauveolens, Pott.
Malvastrum tricuspidatum, A. Gray
‡*Nepeta ruderalis*, Ham.
‡*Nicotiana tabacum*, L.
†*Oplismenus Burmanii*, Beauv.

Oxalis corniculata, L.
†*Paspalidium flavidum*, Stapf.
†*Peristrophe bicalyculata*, Nees.
Phyllanthus niruri, L.
†*Physalis minima*, L.
Ruellia prostrata, Poir.
R. tuberosa, L.
Rungia parviflora, Nees.
‡*Salvia plebefa*, Br.
†*Setaria intermedia*, Roem. and Sch.
†*S. plicata*, T. Cooke.
†*S. rhachitricha*, T. Cooke.
Sida veronicaefolia, Lamk.
Solanum nigrum, L.
S. verbascifolium, L.
†*Triumfetta neglecta*, W. and A.
Vernonia cinerea, Less.

The species marked with † are found in the rainy season and those with ‡ come up in the cold season only.

(e) On annual deposits laid down by the river :

Alhagi camelorum, Fisch.
Alternanthera sessilis, Br.
A. paronychoides, Fort.
Argemone mexicana, L.
Celsia coromandelina, Wall.
Chrozophora Rottleri, A. Juss.
Cochlearia flava, Ham.
Croton sparsiflorus, Morung.
Cynodon dactylon, Pers.
Datura alba, Nees.
Euphorbia spp.

Gnaphalium spp.
Grangea maderaspatana, Poir.
Heliotropium ovalifolium, Forsk.
Lantana indica, Roxb.
✓ *Lippia nodiflora*, Rich.
✓*Mollugo hirta*, Thunb.
✓*Polygonum plebejum*, Br.
Potentilla supina, L.
Rumex dentatus, L.
Veronica anagallis, L.
Xanthium strumarium, L.

3. *Structure and distribution of the communities*

The much eroded and grazed upper ravines bear the *Capparis-Diospyros* associes. The dominant species of the associes are ever-green thorny shrubs which grow upto a height of 10 to 20 feet covered with leaves from the base to the top and are usually infested with a large number of climbers. The community becomes closed in the narrower ravines and the gaps elsewhere are filled up by the ground vegetation which grows very dense during the rainy season when the taller herbs become continuous with the lower shoots of the dominant species. The plateau itself is locally covered by the consocies of *Capparis sepiaria* which, however, is too open due to the intensity of the biotic factors.

The forest association of *Holoptelia-Albizia-Cordia* comes up in the ravines where the *Diospyros-Capparis* associes has minimised grazing and erosion of soil. The dominant trees attain a height of 40 to 60 feet forming a closed canopy at the top which is penetrated by light only in April and May when the leaves are shed off. The second storey of this association consists of young trees and the associes and the consocies of *Capparis* and *Diospyros* on low ridges as these run under the forest canopy. The ridges originally separated the young ravines which have now fused on growing bigger and the neighbouring trees have overtopped them. The lower ravines which get inundated by the river during the rainy season have developed the *Ficus-Pongamia* associes and here societies of *Pongamia glabra* and *Acacia leucophlœa* generally line the association on the Barna side. Where the association is open and sand has been deposited at the bottom of the ravines fragments of a scrub colony of *Acacia arabica, Zizyphus jujuba* and *Phœnix sylvestris* are often met with. They generally follow a consocies of *Saccharum*. These communities would undoubtedly show a better development on the sloping banks of the rivers but for the intense cultivation.

The development of the association is completely arrested at the top of the plateau on account of the biotic factors. There is a small and very open relict consociation of *Holoptelia integrifolia* in the north-east ; the rest of the area is sprinkled with the consocies of *Capparis*.

The herbaceous layer of the association is composed of elements characteristic of situations exposed to erosion, sun and shade and those of the sandy river banks. They show marked seasonal aspects and have already been listed as forming colonies and societies.
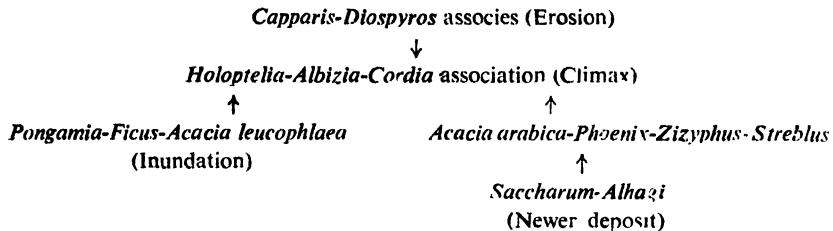
It will be seen that the stratification of the ravine forest association is chiefly on account of a corresponding layering of the soil. The tree layer is supported by the deeper old alluvium, the shrub layer of *Capparis-Diospyros* associes runs underneath on rough lands subjected to monsoon erosion and the ground vegetation is dependent chiefly on the surface soil. *Capparis sepiaria* snd *Diospyros cordifolia* are indifferent to light conditions and since the community maintains its identity with the factor of erosion even under the tree layer it has

been regarded as an independent associes though by structure it forms here only a layer society of the association. Similarly the ground vegetation on the sand, in the lower ravines, has not much to do with the top layers. However, the societies and the colonies dependent on the shade have to be regarded as parts of the association.

The unstable precipice bears patchy colonies of herbs. This is gradually succeeded by the *Capparis-Diospyros* associes which hangs down from the ridge and the species root on the vertical earth. A landslide may rarely cut across the tree covered areas. On the Barna side *Pongamia glabra* and on the sunny Ganges side *Dalbergia sissoo* regenerate more quickly than the other species can do from buds developing on the exposed roots attached to the precipice.

4. *Successional relationships*

The plant communities are developmentally related as shown below :—

<div align="center">

Capparis-Diospyros associes (Erosion)

↓

Holoptelia-Albizia-Cordia association (Climax)

↑   ↑

Pongamia-Ficus-Acacia leucophlaea   *Acacia arabica-Phoenix-Zizyphus-Streblus*

(Inundation)   ↑

Saccharum-Alhagi

(Newer deposit)

</div>

• VI. DISCUSSION

The vegetation of the Rajghat ravines is nearer the climax of the Upper Gangetic Plain which has been described as a monsoon deciduous forest by Dudgeon (1920) and a tropical dry deciduous forest by Champion (1936). In this respect it is very different from the thornscrub type of vegetation consisting of species of *Acacia* or, on saline soils, *Butea frondosa* as is generally obtained in the ravines round about Benares. Erosion of soil and its low capacity for retaining moisture on account of rapid run off and poor plant cover are chiefly responsible for the existence of the thorn-scrub in such areas. But the Rajghat-ravines running down from the plateau and surrounded by the two perpetual rivers actually enjoy better humid conditions and here the old fertile alluvium is more retentive of moisture in spite of the rapid run off. The communities of *Capparis-Diospyros, Saccharum-Alhagi* and *Acacia-Phœnix-Zizyphus* have been shown to be largely dependent on the surface soils and these afford the necessary protection against grazing and thus lead to the development of the deciduous forest association which is sustained by the deeper old alluvium. This association is not large enough here to include many of the constituent species of the climax type as detailed by Champion (1936) for certain places in this province. But it seems to have a relict value fairly indicating that *Holoptelia integrifolia, Albizia lebbek* and *Cordia myxa* are the dominant species of the type for this area.

VII. Summary

The development of the ravines and their vegetation have been described in relation to the environmental factors as obtained at the plateau of Rajghat.

Altogether nine types of plant communities have been recognised. Their structure, distribution and successional relationships are detailed and the nature of the climax vegetation is discussed.

VIII. Acknowledgment

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Dudgeon, W. (1920)   ..   " A Contribution to the Ecology of the Upper Gangetic Plain," *Journ. Ind. Bot. Soc.,* **1,** 1.

Misra, R. (1944)   ..   " An Ecological Study of the Vegetation of Benares Hindu University Grounds" (In press).

# A NEW *ARTABOTRYS* FROM BURMA

BY D. CHATTERJEE

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THE circumstances which led to the discovery of this new plant are interesting. It was first collected by Parkinson in 1929 from tropical rain forests in the Bassein District of Lower Burma. From its resemblance to the common *Artabotrys odoratissimus* R.Br. it was at first thought to be the same. Later it was sent to the Dehra Dun herbarium where it was again identified as *A. odoratissimus*. It was lying under this name in the Forest Herbarium, Maymyo, till 1942 when in the course of a revision of the Burmese Anonaceæ undertaken at Mandalay by the author it was recognised as differing considerably from *A. odoratissimus*. The sheets, together with some others of doubtful determination, were sent to Calcutta by the author shortly before the evacuation of Burma. Later the author was able to have them compared with the Wallichian sheets of *A. odoratissimus* by the kindness of Dr. S. K. Mukherjee, who remarked that "Parkinson's No. 8747 and No. 5060 are very different from Wallich's No. 6418 and these differ amongst themselves and are two distinct species." Parkinson's sheets No. 8747 were sent to Kew for comparison and were returned as "*Artabotrys* not matched ; not *A. odoratissimus* R.Br." Parkinson's No. 8747 is described below. The material of his No. 5060, though sufficient to establish its distinctness, is too scanty for a full description.

Artabotrys Parkinsonii Chatterjee *Sp. Nov.* (Anonaceæ).

Planta distinctissima, Artabotrys odoratissimo habitu foliisque similis, sed ab eo pedunculi floribus minus numerosis minoribusque, petalis obtusis, minutissime pubescentibus, fructu anguste elongato, apice mucronato, inter alia satis recedit.

Extensive climber. Stem and branches terete, brown, longitudinally and minutely wrinkled at least when dry, glabrous, thinly lenticellate. *Leaves* shortly petioled, simple, alternate, lanceolate or elliptic-lanceolate, entire, acute, base cuneate, chartaceous, both surfaces glabrous, upper surface shining ; main nerves thin and rather inconspicuous, 7–9 pairs, spreading and anastomosing in loops by their ends below the margin ; secondary nerves irregularly transverse. Lamina 9–12 cm. long and 3–3.5 cm. wide; petiole short, 3–5 mm., darker than the midrib, shallowly channelled. *Peduncles* usually leaf-opposed, hook flattened, curved, with about 6–8 flowers on each hook (2–3 flowers at the end of first curvature and some 4–5 flowers at the far end); hooks glabrous except near the bases of pedicels which are minutely rufous-tomentose. *Bracts* 2, minute, subulate, rufous-tomentose. *Flowers* regular, bisexual, greenish, 2–2.5 cm. in diam. Pedicel thinly hairy and gradually thickened upwards near the bases of sepals, 1–1·5 cm.
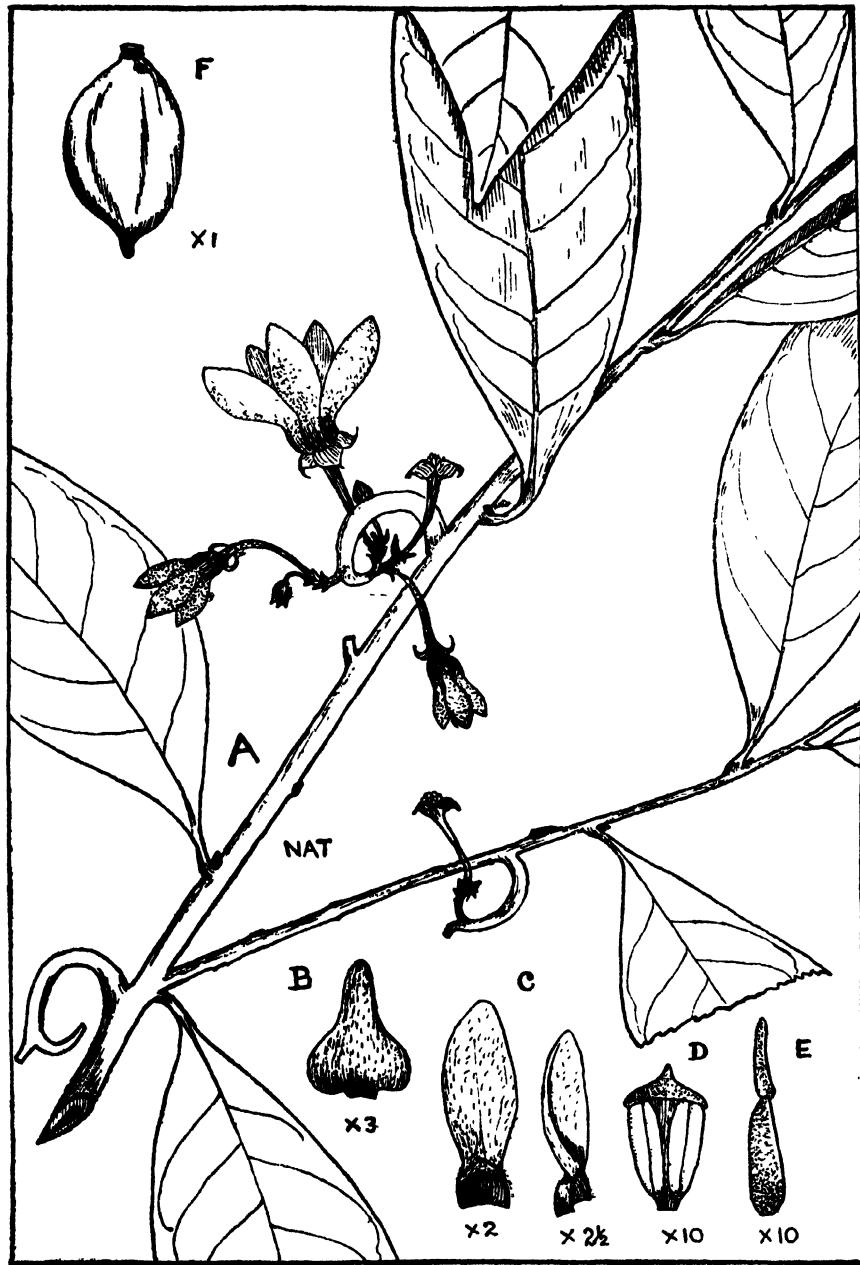
Fig. 1. *Artabotrys Parkinsonii* Chatterjee *Sp. Nov.*
A. Twig with leaves and inflorescence ; B. Sepal ; C. Outer and inner petals ; D. Stamen ; E. Carpel ; F. Fruit.

long. *Sepals* 3, valvate, broadly deltoid, shortly acuminate, coriaceous, 4–5 mm. wide, hairy on both surfaces with golden brown hairs. *Petals* 6, biseriate, free; outer series larger, coriaceous, consrticted near the base, upper part broadly ovate-lanceolate with obtuse apex, both surfaces thinly hairy except the dark and glabrous inner side of the shallow concavity near the base below the constriction, 1.5 cm. long and .5 cm. wide ; a thin, minutely and densely tomentose strip is present just above the glabrous area of the petal below the constriction ; inner petals slightly smaller in size, alternating with the outer, constricted near the base like the outer petals but the concavity at the base is very deep and the inner petal looks like a cochleate lamina; the concavity is dark and glabrous and the limb is thinly hairy; 1 cm. long and .3 cm. wide. *Stamens* many, free, sessile, connective produced to form a cushion-like top with mucronate apex at the centre 2 mm. long, anther-lobes dorsal and narrowly elongated. *Carp ls* many, free, ovary narrowly conical, glabrous; stigma cylindrical, smooth, slightly and gently curved, glabrous, fluted on the top of the ovary where there is a constriction; about 2.5 mm. long with stigma. *Fruit* of 10-12 ripe carpels at the end of the peduncle which is swollen and much thickened ; ripe carpels ellipsoid, dry, subsessile, indehiscent glabrous, with hard mucronate tip. Pericarp rather fibrous, 3 cm. long, 1.7 cm. wide. *Seed* one in each carpel, with ruminate endosperm.

> Burma—Bassein District; Pyinmadon Chaung, *C.E. Parkinson* No. 8747, dated the 15th February 1929 (type and cotype in Herb. Calcutta).

This plant resembles *Artabotrys odoratissimus* in general habit and foliage, but differs in having more flowers on each peduncle, smaller size of the flowers, minutely pubescent obtuse petals, and slightly longer fruit with mucronate hard apex.

ON REDUCTION DIVISION AND AUXOSPORE-FORMATION IN *CYCLOTELLA MENEGHINIANA* KÜTZ.*

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CONTENTS

INTRODUCTION[1]

IT is now fairly well established through the excellent investigations of Klebahn (1896), Karsten (1896, 1897a, 1897b, 1899, 1900 and 1912), Geitler (1927a, 1927b, 1928 and 1932), Cholnoky (1927, 1928, 1929 and 1933a) and Meyer (1929) that auxospore-formation in the Pennales is the result of a sexual process. In the Centrales, on the other hand,

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[1] A preliminary note of this paper was published in *The Journal of the Indian Botanical Society*, 1942. Vol. XXI, Nos. 3 and 4, pp. 231-37.

auxospore-formation is considered to be an asexual or vegetative process and not a sexual one (Oltmanns, 1922 ; Karsten, 1928 ; Hustedt, 1930 ; Smith, 1933 ; and Fritsch, 1935). Again, it has been fairly well ·established that the vegetative phase in the Pennales is diploid, reduction division taking place during auxospore-formation (*loc. cit.*, Klebahn, Karsten, Geitler, Cholnoky, Meyer, Smith and Fritsch), whereas in the case of the Centrales the vegetative phase is generally held to be haploid (Oltmanns, 1922 ; Hustedt, 1930). But some of the recent investigations on the group (Persidsky, 1929, 1935 and Cholnoky, 1933*b*) tend to point out that auxospore-formation in the Centrales also is brought about by a sexual process as in the Pennales and that the vegetative phase even in the Centrales is diploid as in the Pennales, reduction division taking place during auxospore-formation. The number of investigations, however, are so few and the details so meagre that algologists feel that the case for the Centrales is not quite fully established. Fritsch (1935, p. 620) commenting on the observations of Persidsky (1929) on *Chætoceros* and of Cholnoky (1933*b*) on *Melosira arenaria* states : " The possibility of a reduction division and of subsequent autogamy cannot be denied, but further research will be necessary to substantiate this clearly." Geitler (1935, p. 160) states : " Sexual reproduction among the Centrales, with the exception of *Melosira*, is not fully understood. It is probable, however, that the Centrales are also diplonts." Smith (1938, p. 213) when dealing with the Centrales refers to the recent investigations (*viz.*, Persidsky, 1929, 1935 and Cholnoky, 1933*b*) and finally states : " The nuclear behaviour·in the foregoing cases is not established beyond all doubt, but there is a presumption that auxospore-formation is sexual in nature since it involves a fusion of two haploid nuclei. There is also a possibility that auxospores of other Centrales are formed in a similar manner. If this be true, vegetative cells of Centrales are diploid instead of haploid."

The centric diatom *Cyclotella Meneghiniana* Kütz., occurred in plenty at Madras. Advantage was taken of its profuse growth to study its life-history with special reference to the nuclear changes taking place during auxospore-formation.

MATERIAL AND METHODS

Occurrence

The diatom, *Cyclotella Meneghiniana*, was growing in two places at Madras : (1) in a temple tank at Triplicane and (2) in an artificial tank in a garden at Mount Road. It was growing along with various other planktonic algæ such as *Chlamydomonas*, *Scenedesmus*, *Pediastrum*, *Euglena*, *Phacus*, etc., and occurred in plenty from July to October 1940.

Cultures

The diatom was grown in the laboratory in liquid as well as in agar cultures for studying its life-history and cytology.

Liquid cultures.—The method followed by Gross (1937) was used with slight modifications for culturing the present diatom. All the

glass vessels used for the cultures were thoroughly cleaned with a mixture of sulphuric acid and bichromate of potash, washed thoroughly in tap water, then in distilled water and finally dried in a hot air oven (Rawlins, 1933). Petri-dishes were used for the cultures, since the cultures could be easily examined directly under the microscope frequently. Only glass-distilled water uncontaminated by contact with any metal was used for the cultures.

Bacteria-free cultures of the diatom were not aimed at, since the presence of the bacteria in the cultures did not appear to seriously affect the growth of the diatoms. In order to obtain a culture of the diatom as far as possible free from other algæ the following method was adopted. To start with, rough cultures of the diatom were made by picking out the individuals with the aid of a narrow pipette under a Greennough dissecting microscope and transferring them into the culture solution. From the preliminary rough cultures, the diatom cells were again picked out as before with the aid of a narrow pipette under the dissecting microscope and transferred to petri-dishes containing sterilized pond water. From these vessels the diatom cells were again picked out in the same manner and transferred into other petri-dishes containing fresh sterilized water. This process was repeated several times until a fairly pure culture of the diatom was obtained. Finally from this culture the diatom was picked out and transferred to sterilized culture solutions and left growing in them.

For cultures the following two solutions were used : (1) ' Erdschreiber' solution of Föyn (1934) and (2) Allen's (1910) modification of Miquel's solution further modified by Ketchum and Redfield (1938) as per the following formulæ :

(1) ' *Erdschreiber* ' solution

Sodium nitrate	0·1 gm.
Sodium acid phosphate	0·02 gm.
Soil decoction	50 c.c.
Filtered and sterilized pond water	1000 c.c.

(2) *Miquel's solution*

Solution A—

Potassium nitrate	20·2 gm.
Distilled water	100 c.c.

Solution B—

Sodium acid phosphate ..	4 gm.
Calcium chloride	4 gm.
Ferric chloride	2 c.c.
Conc. hydrochloric acid	2 c.c.

Diluted to 100 c.c. with distilled water.

To each litre of sterilized pond water, 0·55 c.c. of solution A and 0·5 c.c. of solution B was added.

For preparing the culture solutions the pond water in which the alga was growing was used. This water was filtered through a porous candle and then sterilized in an autoclave at 120° C. for 20 minutes.

The soil decoction for the ' Erdschreiber ' solution was prepared by adding one kilogram of fine garden soil to one litre of distilled water and heating it in a steam-bath for nearly two hours. On the next day the supernatant liquid was separated and filtered into a flask and then sterilized.

The growth of the diatom in ' Erdschreiber ' solution is very slow to begin with. But the diatom continues to grow in it for several months. In Miquel's solution, on the other hand, the diatom multiplies very rapidly in the beginning, but, if kept in the same medium for a long time, begins to degenerate and die. Even, if reinoculated into fresh solution, it does not show any signs of growth, but ultimately dies. On the other hand, if the diatom, when showing signs of degeneration in the Miquel's solution, is transferred into 'Erdschreiber' solution, it begins to thrive well again and keeps growing in a healthy condition for several months. The following procedure was, therefore, adopted throughout the investigation. The diatom was first grown in Miquel's solution for about 20 days for securing plenty of growth and then transferred into ' Erdschreiber ' solution where it continued to remain in a healthy condition, though growing very slowly.

Agar cultures.—Diatom cells from fairly pure liquid cultures were picked out and pipetted into slightly warm 2% ' Erdschreiber ' agar in test-tubes and, after shaking, dilution cultures were made. The brownish dot-like colonies of the diatom which are formed in these cultures are removed and streaked into fresh agar plates. The diatom multiplies fairly well in these plates and often assumes a filamentous condition (*cf.* below).

For cytological studies material from the cultures was fixed during the· twenty-four hours of the day at intervals of one hour each. Material freshly collected from the field was also fixed in the same manner. The most abundant cytological stages were obtained in material fixed between 5 A.M. and 9 A.M.

The following fixing fluids were used :—Fleming's weak formula, Schaudinn's sublimate-acetic-alcohol (acetic acid 5%), Allen's modification of Bouin's fluid (P.F.A₃). Of these hot Schaudinn's solution and P.F.A₃ gave the best results. The material in both cases was left in the fluid for 5 to 12 hours and then washed, the washing being done with the aid of a centrifuge. The Schaudinn material was washed several times in 50% alcohol first, and then in 70% alcohol. Traces of mercuric chloride were finally removed by treatment with Lugol's iodine solution. The Bouin material was washed in a few changes of 50% alcohol and then in 70% alcohol and the traces of picric acid that may still remain were finally removed by the addition of a few drops of saturated solution of lithium carbonate in 70% alcohol. After washing, the material was preserved in 70% alcohol.

Smear preparations were also made by killing the material on slides previously smeared with a thin coating of Mayer's albumen as per proto-zoological methods (McClung, 1937, pp. 530–31).

The following stains were employed :—Heidenhain's iron-alum hematoxylin, safranin, Mayer's hæmalum, Newton's gentian violet and

safranin and light green. Iron-alum hematoxylin gave the best results. Good results were also obtained by counterstaining the hematoxylin preparations with ½% erythrosin in 95% alcohol.

The procedure adopted for staining in iron-alum hematoxylin is as follows :—A small quantity of the material preserved in 70% alcohol was spread on slides previously smeared with a thin coating of Mayer's albumen and, before the material dried completely, the slide was placed in 85% alcohol. The slight coagulation of the albumen helps to fix the material firmly to the slide. The slides were then brought down the alcohol grades to 30% alcohol and then bleached in 30% alcohol containing 10% hydrogen peroxide. Bleaching in chlorine is not suitable since the material drops away from the slide. The slides were kept in this for 30 minutes and then washed thoroughly in running water for 15 minutes. Bleaching gave a better contrasted stain.

After washing, the material was mordanted in 4% iron-alum solution for one hour, washed in running water for 15 minutes, stained in ½% hematoxylin for 6–12 hours and differentiated in saturated solution of picric acid. The slides were next washed overnight in running water and passed through the alcohol grades into absolute alcohol, then into clove oil and then into xylol and finally mounted in neutral canada balsam dissolved in xylol.

For counterstaining, the slide from 95% alcohol was placed in a ½% solution of erythrosin in 95% alochol for about 10 seconds and passed rapidly into absolute alcohol and clove oil, cleared in xylol and mounted in neutral canada balsam.

Material stained for 3 hours in Mayer's hæmalum diluted to about one-fourth the strength and washed overnight in running water also gave good preparations. This did not require any differentiation.

DESCRIPTION OF THE CELL

The cells of *Cyclotella Meneghiniana* (Pl. VIII, Fig. 1) are disc-shaped in valve view (Text-fig. 1) and rectangular in girdle view (Text-fig. 2) with an undulation on each of the long sides. In valve view the margin appears striated radially (Pl. I, Fig. 1). The cytoplasm forms a thin lining layer close to the wall and the nucleus is either embedded in this lining layer of cytoplasm or remains suspended by cytoplasmic strands at the centre of the cell. The chromatophores which are lobed or disc-shaped are yelowish-brown in colour (Text-figs. 1 and 2).

VEGETATIVE MULTIPLICATION

The diatom multiplies by successive division. Division generally takes place in the early hours of the morning.

Somatic mitosis

The resting nucleus (Text-fig. 1) measures about 4μ in diameter and has generally one nucleolus. It enlarges slightly prior to division. The nucleus during division is generally situated in the parietal layer of cytoplasm. Cholnoky (1933*b*) also found a movement of the nucleus towards one side during division in *Melosira arenaria.*
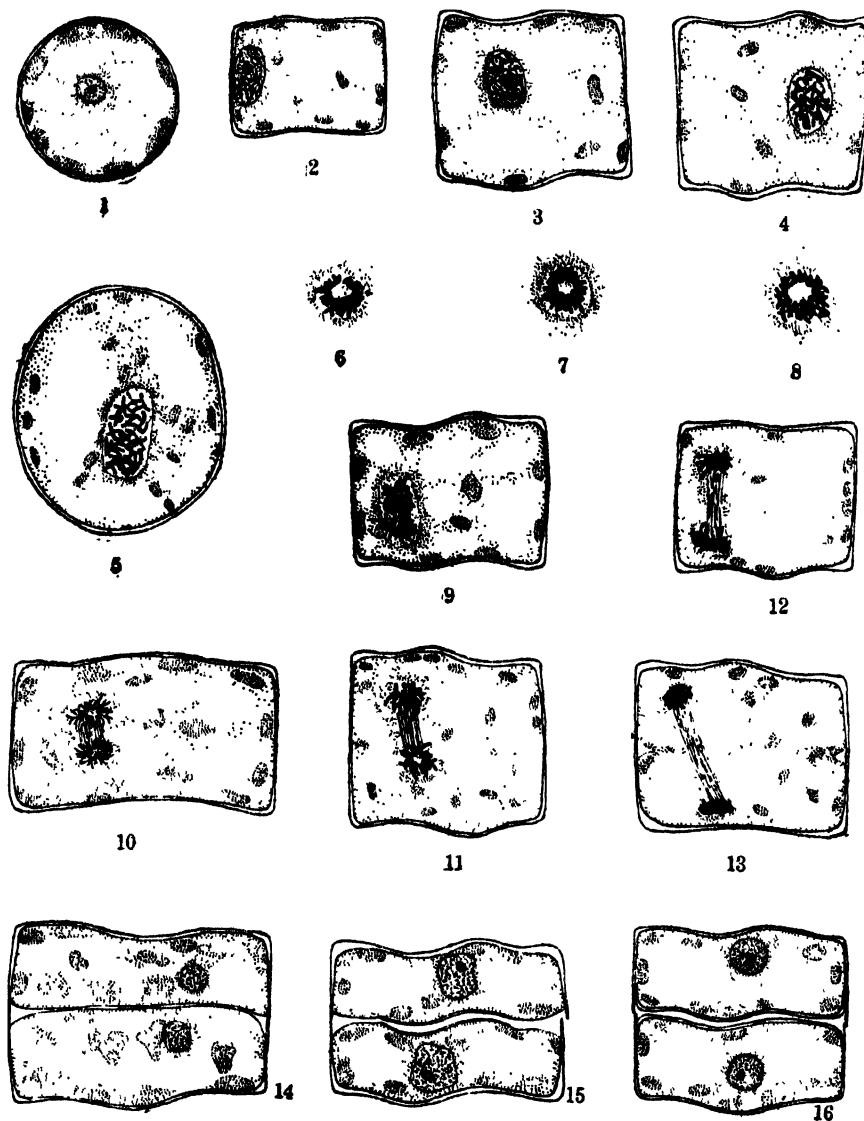
Text-figs. 1–16. *Cyclotella Meneghiniana* Kütz.—Fig. 1. Resting nucleus. Cell in valve view. (×1120). Figs. 2 and 3. Early prophase. Cells in girdle view. Fig. 2 (×1120); Fig. 3 (×1520). Fig. 4. Late prophase. Cell in girdle view: the nucleolus already disappeared (×1520). Fig. 5. Late prophase. Cell in valve view (×1520). Figs. 6–8. Metaphase, polar view. Note arrangement of chromosomes in a ring around the spindle. In Fig. 6, the gap in the chromosome ring is clearly seen. Note spindle seen in the polar view as dots inside the chromosome ring (×1520). Fig. 9. Early anaphase (×1520). Fig. 10. Early anaphase. Note peculiar dark body on the spindle (×1120). Figs. 11–12. Late anaphase

(×1520).   Fig. 13.   Early telophase.   Note beginning of cytokinesis (×1520)..
Fig. 14.   Division of cytoplasm completed (×1120).   Figs. 15–16.   Formation of
new valves.   Figs. 15–16.   Formation of new valves ; in Fig. 15 beginning of valve
formation seen at the central portion : in Fig. 16, the valve formation is complete
(×1120).

In the resting nucleus the reticulum takes a light stain.   The
chromatin granules could be seen rather sharply stained in the reticulum
(Text-fig. 1).   In early prophase the chromosome threads are thin and
long (Text-figs. 2 and 3).   In late prophase they become somewhat
contracted and thicker and are seen lying in the nuclear cavity (Text-
figs. 4 and 5).   The nucleolus which is visible up to this stage
disappears completely now (Text-figs. 4 and 5).

In metaphase the chromosomes are seen arranged in a ring around
the equator of the spindle (Text-figs. 6, 7 and 8).   The side view of
metaphase was not observed.   Only the polar view was obtained in the
preparations.   In the polar view, the fibres of the spindle could be
seen as a number of dots at the centre of the chromosome ring
(Text-figs. 6–8).   At this stage no trace of the nuclear membrane is
evident, though a clear space is seen in its place.   The chromosomes
are rod-shaped, V-shaped and V-shaped with unequal arms (Text-
figs. 6–8).   The chromosome number could not be definitely determined
owing to their large number and very small size and their very
compact arrangement in the ring.   The number appeared to be more
than 60.

The anaphase figures are characterised by long trailing chromo-
somes which lie more or less parallel to the axis of the spindle
(Text-figs. 9 and 11).   The chromosomes after reaching the poles of the
spindle contract and become compacted together (Text-figs. 12 and 13).
In properly differentiated preparations, the outline of a few chromo-
somes could still be seen in the clumps. In one single instance a darkly
stained body was observed in anaphase lying between the two groups
of daughter chromosomes on the spindle (Text-fig. 10).   The nature
of this body could not be determined.

In telophase (Text-fig. 14) the chromosomes gradually become
reorganised into the reticulum   (Text-figs. 15 and 16).

Cytokinesis

Cytokinesis takes place by the furrowing of the cytoplasm.   At
about anaphase, a small cleavage furrow is seen starting from the two
sides in the girdle view (Text-fig. 13).   This cleavage furrow advances
inwards in a centripetal manner.   During telophase, the furrow advances
still further inwards and finally cuts through the spindle fibres and
cytokinesis becomes complete.   After cytokinesis is completed, the two
daughter nuclei are seen very close to each other.   Each of the two
daughter protoplasts then secretes a new valve on its inner side which
fits inside that of the respective mother valve (Text-figs. 15 and 16).
The two cells separate soon after.

A small point may be mentioned in this connection.   Prior to
division, the cells seem to increase in volume by the valves loosening
slightly to allow for the increase.   Cholnoky (1933*b*) also has recorded

a similar increase in the volume of the cell prior to division in *Melosira arenaria*.

In agar cultures, owing to the daughter cells not separating from each other soon after cell-division, the diatom assumes a filamentous condition, which, however, is soon lost when it is transferred to a liquid medium.

AUXOSPORE-FORMATION

There appear to be so far only very few records of auxospore-formation in the genus *Cyclotella*. The first record of auxospore-formation in this genus appears to be by Thwaites in *C. Kützingiana* (Thwaites, 1848, p. 166, 169, pl. XI, fig. D. 1–5), and later on by W. Smith (1856, vol. II, p. x, pl. B, fig. 47, I–IV) in the same diatom. Hofmeister observed auxospore-formation in *C. operculata* Kütz. in 1857. Later Miquel (1891–92) recorded auxospore-formation in *C. compta* and Bachmann (1904) in *C. bodanica* var. *lemanica*. Hustedt (1930, p. 100) states that auxospores are formed in *C. Meneghiniana* but does not give any details. In all these cases the observations regarding auxospore-formation were very meagre and superficial and no cytological details were attempted.

In the case of the present diatom, plenty of auxospore-formation was observed in the laboratory cultures. An intensive study of the auxospore-formation was made and a detailed account of this process is given here below.

The diatom when inoculated into the culture medium (liquid or agar medium) shows a period of rapid multiplication by vegetative division. This rapid multiplication lasts only for a short time and soon the rate of multiplication slackens and the diatom finally stops dividing altogether. If, at this time, the culture medium is changed, the diatom divides rapidly again for some time and then again stops multiplying. If it is again changed into a fresh culture medium, it begins to divide again rapidly. In this way, repeated transference to fresh culture media helps to keep up the multiplication of the diatom. But, soon there comes a stage when the diatom does not any further respond to changes of the culture media, and for days together no further increase of cells could be noticed, and the diatom begins to die gradually. But, if the diatom cells, when beginning to deteriorate, are transferred to sterilized pond water or to sterilized pond water slightly diluted to about 10–20% with sterilized distilled water, they continue to live healthily and do not degenerate. They do not, however, show any division. At this stage, after some time, they begin to show auxospore-formation. This auxospore-formation was more common in the diluted pond water than in the undiluted pond water. But, only a slight dilution (*i.e.*, 10–20%) is favourable for auxospore-formation. For, if the pond water is diluted to more than 20% with distilled water (say 30%, 40% and 50%), the diatom degenerates and dies out rapidly.

It may be mentioned in this connection that some of the previous workers also found that, when the cells have reached a minimum size,

a slight dilution of the culture medium induced auxospore-formation. Schreiber (1931) observed in the case of *Melosira nummuloides* that the cells which have reached a minimum size, when transferred to sea water of a slightly lower concentration, formed auxospores, but, when transferred to sea water of a higher concentration, exhibited abnormalities. He also found that such of the cells as had not yet attained the minimum size, did not form auxospores when transferred to a medium of lower concentration. Geitler (1932, p. 194) found in the case of *Navicula seminulum* that transference to a medium of higher concentration definitely hindered auxospore-formation. He also found in his investigations on several pennate diatoms that the cells formed auxospores only after attaining a certain minimum size. Gross (1937–38 pp. 25, 26 and 45) found that if the cells of *Ditylum Brightwellii* after reaching a certain size are transferred from sea water ' Erdschreiber ' solution to pure sea water (which is a medium of slightly lower concentration), they formed auxospores. Earlier Cholnoky (1928, p. 26) had noticed in the case of *Anomœoneis sculpta* E-Cl. (Pennatæ) that a sudden decrease in the concentration of the medium brought about auxospore-formation. He, however, does not mention anything regarding the size of the cells when forming auxospores.

It was mentioned above that the diatoms form auxospores only after reaching a miniumm size. The same phenomenon was observed by the previous workers also. Pfitzer (1871, pp. 155–56) states that in the case of several diatoms a minimum size should be reached for auxospore-formation. Bachmann (1904) found the same thing in the case of *Cyclotella bodanica* var. *lemanica.* As mentioned already Schreiber (1931), Geitler (1932, 1935) and Gross (1937–38) also found the same thing in the forms investigated by them.

In the present diatom the minimum size for auxospore-formation is generally 10–15 μ in diameter, *i.e.,* about one-third the maximum size of the diatom. But, it was rather peculiar that all the individuals which have become smaller than this size (*i.e.,* below 10 μ in diameter) did not form auxospores but, degenerated and died. In this connection it may be mentioned that Geitler (1932, 1935) also found in the case of several Pennate diatoms that individuals which become very small in size generally died and did not take any further part in the life-cycle.

Description of the process in the living material

In *Cyclotella Meneghiniana* auxospore-formation takes place in the early hours of the morning. The two valves move apart with a slight jerk, which could be seen very clearly when the diatom is watched under the microscope (Text-fig. 17). This sudden jerk is probably brought about by a rapid increase in the turgor pressure inside the cell. After the valves have thus moved apart, the protoplast gradually emerges out from the valves (Text-figs. 18–20 ; Pl. VIII, Fig. 2). When emerging out, it is seen surrounded by the perizonium, the inner pectic layer of the diatom (Text-figs. 21, 22 ; Pl. VIII, Fig. 2). During this process the protoplast escapes out of one of the valves first and then from the other valve (Text-figs. 18–20). As it comes out,
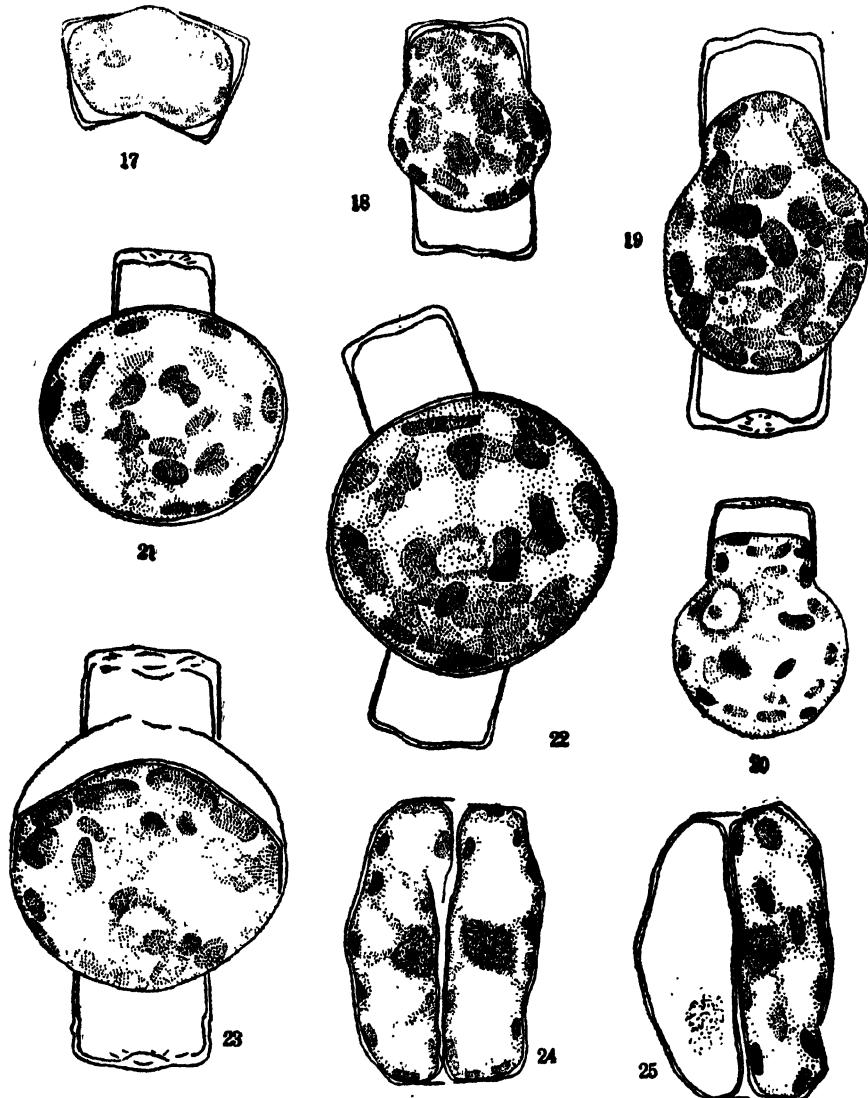
Text-figs. 17–25. *Cyclotella Meneghiniana* Kütz.—Figs. 17–23. Auxospore-formation as seen in the living specimens. Fig. 17. Valves just pushed apart by the enlarging protoplast (×850). Figs. 18–20. Protoplast emerging out of the valves and enlarging during the process. Note the contents emerging out of one of the valves first. In Fig. 20, one valve has dropped off. Figs. 18 and 19 (×1120); Fig. 20 (×850). Figs. 21–22. The enlarged protoplast completely outside the valves and surrounded by the perizonium. (The second valve has already dropped off in Fig. 21.) Fig. 21 (×850); Fig. 22 (×1120). Fig. 23. Formation of the first valve (epitheca) inside the perizonium (×1120). Figs. 24–25. Two daughter cells which were formed after the first division of the new cell. Note the degeneration of one of the two daughter cells in Fig. 25 (×1120).

it enlarges gradually and finally becomes very much swollen with the two valves attached to it at the opposite sides (Text-figs. 19–22 ; Pl. VIII, Figs. 2, 3). Occasionally one of the valves may be seen a little displaced to one side (Text-fig. 22 ; Pl. VIII, Fig. 3) or even dropping off (Text-figs. 20, 21). The chromotophores are distributed near the periphery of the swollen protoplast.

The swollen protoplast then contracts from one side of the perizonium (viewed from the girdle view of the mother valve) and secretes a curved siliceous valve, the epitheca (Text-fig. 23 ; Pl. VIII, Fig. 4). It then contracts from the opposite side also and secretes a second valve, the hypotheca, which fits into the epitheca. The characteristic markings of the diatom soon become evident on the two valves. The cell when completed is situated in the middle of the perizonium, its two valves being generally more or less parallel to those of the old auxospore-mother-cell valves. In girdle view the cell is somewhat plano-convex, the epitheca being more convex than the hypotheca, which shows the characteristic undulation of the diatom (Text-fig. 57). The perizonium finally gets ruptured and the new cell is liberated (Text-fig. 57). The whole process of auxospore-formation takes about five hours to complete.

The new cell has a diameter varying from 38–45 μ. This is about three times the diameter of the auxospore-mother-cell. This ratio of the diameter of the original mother-cell to that of the new cell agrees with that recorded by Müller (1906) for *Melosira italica* (Fritsch, 1935, p. 620), by Schütt (1889) for *Chætoceros* and Schulz (1930, p. 28) for *Thallassiosira baltica* (Grun.) Ostenfeld.

Nuclear Changes accompanying Auxospore-formation

The resting nucleus of the cell which is to give rise to an auxospore has a well defined nuclear membrane, a lightly staining reticulum and a single nucleolus. This nucleus is in no way different from that of the ordinary vegetative cell in appearance. It divides twice and forms four nuclei. The first of these two divisions is a reduction division. During the prophase of this reduction division the nucleus increases to almost double its normal size (Text-fig. 26). Thin chromosomal threads become discernible in the nucleus (Text-figs. 26–28). At the next stage (synizesis) the threads are seen more contracted and thicker and lying on one side of the nuclear cavity (Text-fig. 29 ; Pl. VIII, Fig. 5). The free ends of several chromosomal threads are clearly visible and their paired nature can be made out on careful examination. In the next stage (pachytene) the paired chromosomes are seen distributed more uniformly in the nucleus and are thicker and shorter than in the previous stage (Text-fig. 30, 31 ; Pl. VIII, Fig. 7). The bivalents then become still shorter and exhibit their paired nature very clearly (Text-fig. 32 ; Pl. VIII, Fig. 6). The next stage observed in the preparations was diakinesis (Text-figs. 33–35, 36–38 ; Pl. VIII, Figs. 8, 9 and 10). During this stage the bivalents are seen distributed near the periphery of the nucleus. The number of bivalents appears to be about 32–34 (n). The nuclear membrane and the nucleolus disappear soon after diakinesis (Text-figs. 33–38 and 39).
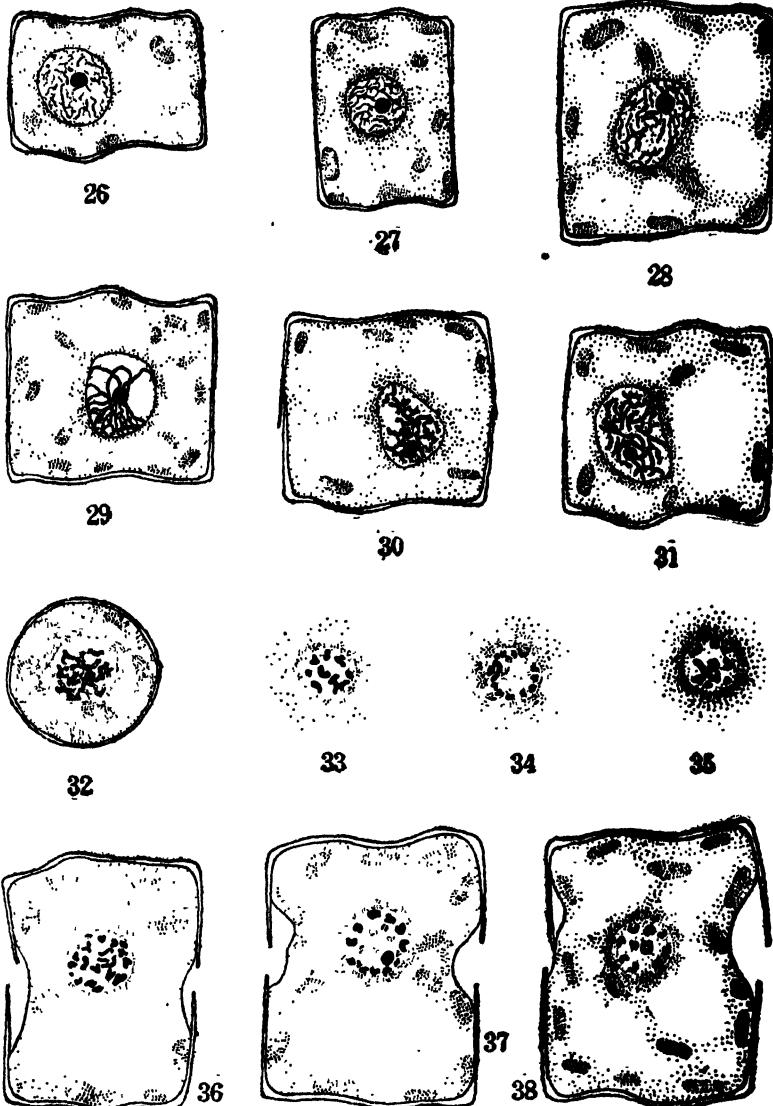
Text-figs. 26-38. *Cyclotella Meneghiniana* Kütz.—Figs. 26-28. Early prophase of reduction division (× 1400). Fig. 29. Synizesis (× 1400). Figs. 30-32. Pachytene (× 1400). Figs. 30 and 31 in girdle view ; Fig. 32, in valve view. Note the much shortened and thickened bivalents. Figs. 33-35. Diakinesis in three different foci : Fig. 33, in upper focus : Fig. 34, in median focus and Fig. 35, in lower focus. Nucleolus seen in Fig. 35. In Figs. 34 and 35, one of bivalents is still uncontracted. (× 1400). Figs. 36-38. Diakinesis in another cell in three different foci : Fig. 36 in upper focus ; Fig. 37, in median focus : and Fig. 38, in lower focus. Nucleolus, seen in Fig. 37 (× 1400).

During metaphase the bivalents are seen very compactly arranged in a ring around the spindle (Text-fig. 40 ; Pl. VIII, Fig. 11).   After anaphase (Text-fig. 41 ; Pl. IX, Fig. 12) and telophase (Text-fig. 42) two daughter nuclei are organised (Text-fig. 43).   The two nuclei next enter into the homeotypic division either simultaneously (Text-figs. 44–45 ; Pl. IX, Figs. 13 and 14) or successively (Text-figs. 46–48 ; Pl. IX, Figs. 15 and 16) and form four nuclei. Persidsky (1935) found that in *Melosira varians* also one of the two nuclei sometimes divided later than the other in the second division.

By the time the homeotypic division is completed, the protoplast with the four nuclei emerges outside the valves and becomes very slightly increased in size.   Two of the four nuclei degenerate and the remaining two are normal and healthy.   Text-figs. 49 and 50, and Pl. IX, Fig. 17 show two healthy nuclei and two degenerating nuclei. Each of the two degenerating nuclei appears as a darkly stained mass. It may be mentioned in this connection that no case was observed in which all the four nuclei were normal.   In every case observed, two of the four nuclei had already degenerated.   The degeneration of the two nuclei presumably sets in very early after the second division, since in all the cases observed two nuclei had already degenerated. In one case, during second division, one of the groups of daughter chromosomes in each of the division figures appeared to be smaller and more compact than the other (Text-fig. 45).   Whether this has any significance in relation to the early degeneration of the two nuclei could not be determined with certainty.   It is just possible that the smaller and more compact group of chromosomes in each of the two division figures represents the degenerating daughter nucleus.   The healthy nuclei then approach each other and lie in close contact with each other (Text-fig. 50 ; Pl. IX, Fig. 17) and then ultimately fuse (Text-fig. 51 ; Pl. IX, Fig. 18).   These two nuclei according to Geitler (1932, p. 10) form the ' gametic nuclei '.   Each of these two gametic nuclei always shows a single nucleolus (Text-figs. 49 and 50 ; Pl. IX. Fig. 17).   The fusion nucleus[2] shows two nucleoli (Text-figs. 52–55 ; Pl. IX, Figs. 19 and 21) and this condition is seen even after the formation of the valves (Text-figs. 56 and 57).   This fusion nucleus forms the nucleus of the new diatom cell formed by the auxospore.

It may be mentioned here that the two gametic nuclei at the time of fusion are in early prophase (Text-figs. 50, 51).   The fusion nucleus immediately after fusion is likewise in prophase condition (Text-figs. 52–55).   This is in agreement with the observations of Cholnoky (1928, 1929, 1933a) on several pennate diatoms, where he found that the gametic nuclei were in prophase condition prior to fusion and so also was the fusion nucleus immediately after fusion.   In another diatom, *Melosira arenaria*, one of the Centrales, he (1933b) found a large nucleus in prophase during auxospore-formation and presumed that it must have resulted from the fusion of two gametic nuclei.   It may be mentioned here that even in the case of some higher plants

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[2] ' Syncaryon ' (Geitler, 1935, p. 159).   ' Copulatory nucleus ' (Persidsky, 1935, p. 129).

the nucleus of one or both the fusing gametes has been known to attain a prophasic condition during fusion (Sharp, 1934, pp. 236–37).
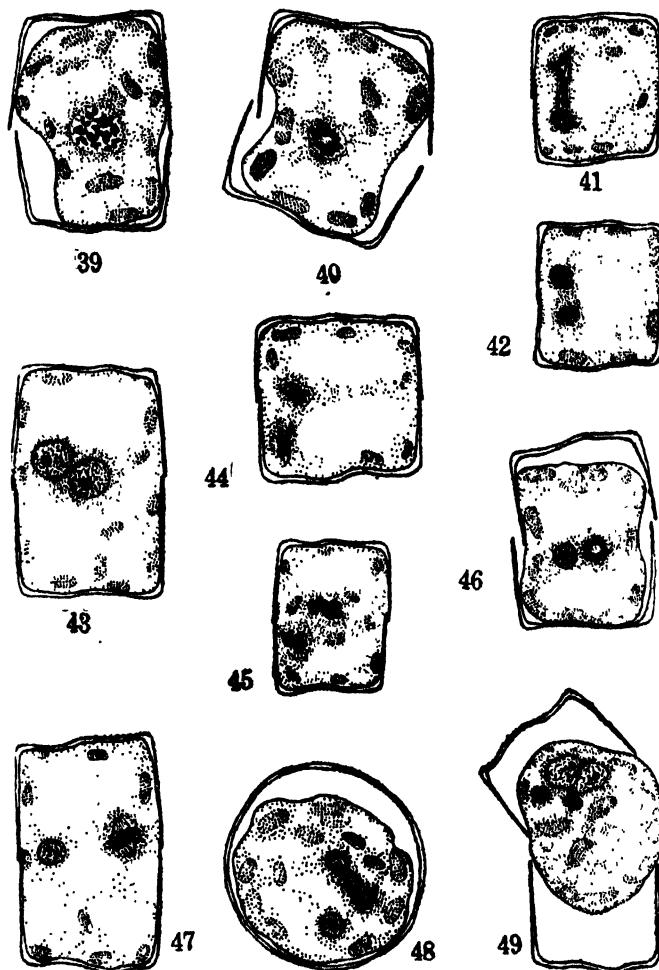
Text-figs. 39–49. *Cyclotella Meneghiniana* Kütz.—Fig. 39. Late diakinesis. All the bivalents shortened. Note nucleolus and nuclear membrane disappeared (×1400). Fig. 40. Metaphase of I division. Note chromosomes arranged in a ring (×1400). Fig. 41. Anaphase (×1400). Fig. 42. Telophase (×1400). Fig. 43. Two-nucleate stage after I division (×1400). Fig. 44. II Division early anaphase. Note both nuclei in division (×1400). Fig. 45. II division anaphase. Both nuclei in division (×1400). Fig. 46. II division. One nucleus in prophase and the other in metaphase (×1400). Fig. 47. II division. One nucleus in early anaphase and the other in prophase (×1400). Fig. 48. II division (valve view). One nucleus in resting condition and the other in late anaphase (×1400). Fig. 49. Four-nucleate stage with two normal and two degenerating nuclei (dark bodies). Note the prophase condition of the two functional nuclei (×1400).

The auxospore in the meantime enlarges to its full extent with the mother valves clinging to the perizonium (Text-figs. 53–55 ; Pl. IX, Fig. 22). The increase in size of the auxospore takes place only after the fusion of the gametic nuclei, the fusion probably acting as a stimulus. Persidsky (1935, p. 129) also noticed that the same was the case in *Melosira varians.*

The two degenerating nuclei gradually become paler and ultimately disappear completely. In some cases they persist for some time even after the formation of the new valves (Text-figs. 56, 57).

DEVELOPMENT OF THE NEW CELL RESULTING FROM
AUXOSPORE-FORMATION

The cell resulting from auxospore-formation is, as already mentioned, somewhat plano-convex in girdle view, the side of the epitheca being slightly convex while that of the hypotheca more or less flat though somewhat wavy (Text-fig. 57). During the division of this cell two new valves with more or less flat sides are inserted inside the old valves with the result that one of the daughter cells (the one which inherits the old epitheca) is plano-convex (Text-figs. 24, 25) while the other (the cell which gets the old hypotheca) is flat on both sides like a normal individual (Text-figs. 24, 25). Two instances of such a division were observed in the material. In one of these, the daughter cell which had inherited the old epitheca was found degenerating, while the other cell was quite healthy (Text-fig. 25). In the other instance both the daughters were quite healthy (Text-fig. 24). Whether the plano-convex daughter,cell which is healthy in this case will later on degenerate, it is not possible to state.

The failure of one of the daughter cells of an auxospore to develop normally has been recorded by Geitler (1932, 1935) in the following two members of the Pennales. In *Cocconeis* sp. he found that the first division of the auxospore produces two unequal daughter cells, one of which develops normally, while the other is always irregular in a definite manner and possesses no raphe. The latter is not capable of further development. In *Cymbella sumatrensis,* vegetative nuclear division in the auxospore takes place without the division of the cell contents and one of the daughter nuclei disappears while the other becomes the nucleus of the auxospore which develops normally. With regard to this Geitler (1935, p. 156) states : " An accurate interpretation of this process is not yet possible ; it may be supposed, however, that a preliminary rudimentary cell-division does take place which is represented by the surviving nuclear division ". Such instances, where one of the daughter cells fails to develop do not appear to have been recorded so far among the centric diatoms.

DISCUSSION

Thwaites (1848, p. 166) who is probably the first to study the process of auxospore-formation in the Centrales considers that it is probably a sexual process and states that " there is a great probability of a process taking place in the one cell of the *Melosireæ* precisely
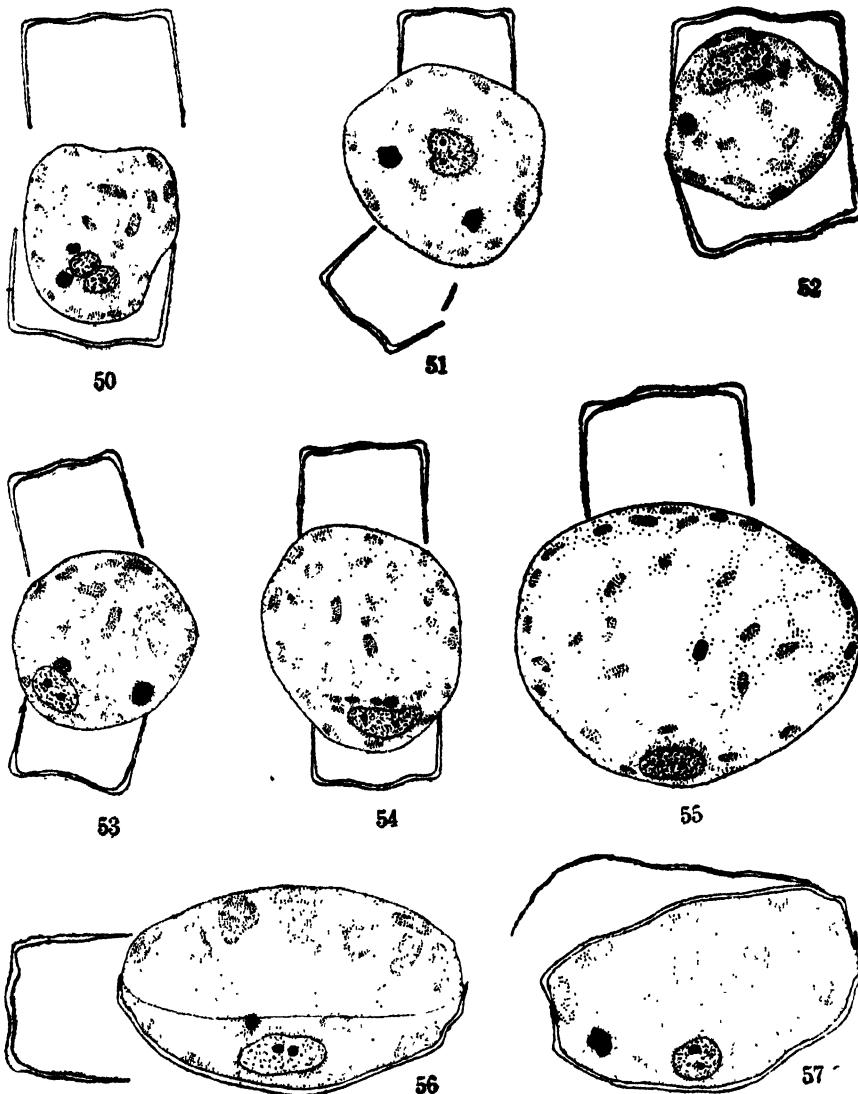
Text-figs. 50–57. *Cyclotella Meneghiniana* Kütz.—Fig. 50. Four-nucleate condition. Same as Fig. 49 (×1240). Fig. 51. Fusion of the two functional gametic nuclei. The dark bodies represent the degenerating nuclei. Note prophasic condition of the fusing nuclei (×1240). Fig. 52. Fusion of the gametic nuclei completed ; fusion nucleus with two nucleoli. Note the prophasic condition of the fusion nucleus (×1240). Figs. 53–55. Protoplast in different stages of enlargement. Note fusion nucleus in all with two nucleoli. In Fig. 55 only one degenerating nucleus seen (×1240). Fig. 56. Formation of the epitheca. Note arched nature of the valve. The mother valve displaced from the usual position. Note also the nucleus with two nucleoli and one degenerating nucleus. Fig. 57. New cells with both valves formed and remnant of the perizonium sticking to it (×1240).

similar in physiological character to the conjugation or mixture of endochromes in other species ". He comes to the conclusion mainly on a conjectural basis without any evidence in support of his conclusion (Pfitzer, 1871, pp. 130–31). He states that auxospore-formation in *Cyclotella Kützingiana* (Thwaites, 1848, p. 169) and in the *Biddulphieæ* (*ibid.*, p. 166 foot-note) is quite similar to that of the *Melosireæ*. Other authors following Thwaites, such as Braun (1851), Smith (1856), de Bary (1855–57) and Lüders (1862), supported his interpretation of the process (Pfitzer, 1871, p. 131).

But later authors thought that the process was not sexual but purely asexual or vegetative. Pfitzer (1871, p. 131) who investigated auxospore-formation in *Melosira varians* considered it a purely vegetative process since the auxospore was formed from a single individual.

Müller (1889) who studied auxospore-formation in *Terpsinoë musica* states that it is not a sexual process but only a simple case of rejuvenation of the cell. According to him, auxospore-formation in this diatom takes place in the same manner as the one described by Pfitzer (1871) for *Melosira varians*.

Karsten (1897*b*, pp. 216–17) found that the nucleus of the young auxospores of *Melosira nummuloides* and *M. moniliformis* (*M. Borreri*) contained two nucleoli whereas the nucleus of the vegetative cells had only one nucleolus. He recorded a similar phenomenon in *Sceletonema costatum* (Karsten, 1897*b*, p. 218) also. He supposed that a rudimentary division takes place during auxospore-formation.

Bachmann (1904) who studied auxospore-formation in *Cyclotella bodanica* var. *lemanica* agreed with Karsten in thinking that a supression of cell division takes place during auxospore-formation. He states that, due to external and internal conditions, a sudden increase of turgor pressure occurs leading to auxospore-formation, as a result of which the nuclear division which begins is interrupted, and the cell division is suppressed.

Yendo and Akatsuka (1910) described the auxospore-formation in *Arachnoidiscus Ehrenbergii* as asexual.

Hustedt (1923, 1930) states that the formation of auxospores in *Melosira Jürgensi* Ag. and *M. arenaria* Moore is an asexual process (Hustedt, 1930, p. 115) and states that in *M. Jürgensi* during auxospore-formation there is a suppression of nuclear division as suggested by Karsten.

While all the investigations up to this period have taken it for granted that auxospore-formation in the Centrales is only a vegetative or asexual process, a few very recent investigators (from 1929 onwards) on the Centrales have brought out evidence suggesting that the Centrales are not very different from the Pennales as regards their mode of auxospore-formation.

Persidsky (1929) investigated auxospore-formation in two species of *Chætoceros*, viz., *Ch. boreale* and *Ch. densum*. He found that the nucleus of the auxospore-mother-cell divides twice and forms four nuclei. Of the four nuclei that are formed, two fuse and form the

nucleus of the auxospore, while the remaining two degenerate. He considers the first division as heterotypic, since he claims to have found both "synapsis" and diakinesis stages during this division. Geitler (1931, p. 9) considers the figures given by Persidsky of these stages as quite unconvincing (*cf.* Geitler, 1931, p. 8, figs. 6 *a* and *b*), but considering the similarity of these stages represented by Persidsky to those in the Pennales, he thinks that Persidsky's explanation is probably correct.

Schmidt (1930, pp. 459–61) criticises Persidsky's paper stating that Persidsky was not able to see all the stages continuously. And, he states that none of the earlier workers were able to observe any such nuclear changes during auxospore-formation.

Cholnoky (1933*b*) found in the young auxospores of *Melosira arenaria* one large nucleus and two small degenerating nuclei. The large one becomes the nucleus of the auxospore. He presumes that reduction division takes place before auxospore-formation and that out of the four nuclei that are formed, two fuse and form the large nucleus of the auxospore and the other two degenerate.

Geitler (1934, p. 423) mentions that he found in the auxospores of an undetermined species of *Melosira* one functioning nucleus and one or two degenerating nuclei.

In 1935, Persidsky investigated auxospore-formation in another centric diatom, *viz., Melosira varians.* In this he found that during auxospore-formation the nucleus of the mother-cell undergoes two successive divisions and forms four nuclei. Two of these four nuclei fuse and form the nucleus of the auxospore, while the remaining two degenerate. Of these two nuclear divisions again, he found that the first is definitely heterotypic though he was able to observe only a few stages like synizesis, late anaphase and telophase. The figures given by him in support of the reduction division in this case are more convincing than the ones he gave in support of the reduction division in *Chætoceros* (Persidsky, 1929). Thus the occurrence of reduction division and the fusion of the two nuclei which appeared to be a little doubtful in the case of *Chætoceros* were definitely proved by him to be actual facts in the case of *Melosira varians.* This investigation lends full support to Cholnoky's (1933*b*) and Geitler's (1934) earlier observations mentioned above.

Gross (1937–38) found in the auxospores of *Ditylum Brightwellii* (West) Grun. one large nucleus and two smaller ones and interprets his observations in the same way as Cholnoky (1933 *b*) did in the case of *Melosira arenaria.*

Reith (1940) found during auxospore-formation in *M. arenaria* a large nucleus and a degenerating residual nucleus and states that his observations correspond with those of Cholnoky (1933*b*) on the same diatom.

The above-mentioned recent investigations suggest (1) that auxospore-formation in the few members of the Centrales that have been investigated is the result of a sexual process as in the Pennales (though

through the autogamous fusion of two gametic nuclei) and (2) that the vegetative cells are diploid as in the Pennales and undergo reduction division during auxospore-formation.

The observations made by the authors on the present diatom, *Cyclotella Meneghiniana*, are in full agreement with those of Persidsky (1935) on *Melosira varians*. The vegetative phase in the present diatom is definitely diploid. During auxospore-formation the nucleus undergoes two divisions and forms four nuclei. Of these two nuclear divisions the first is definitely meiotic. In this first division almost all the characteristic stages of the meiotic division have been clearly observed. Of the four nuclei that are formed two fuse and form the nucleus of the auxospore, while the remaining two degenerate. Auxospore-formation here is clearly the result of a definite sexual process brought about through the autogamous fusion of two gametic nuclei.

It was mentioned above that Karsten (1897b) recorded in the nucleus of the young auxospores of *Melosira nummuloides* and *Melosira moniliformis* two nucleoli and interpreted his observation as indicating a suppression of nuclear division. Geitler (1932, p. 201) criticises Karsten's interpretation and states that the presence of two nucleoli in the young auxospores could on no account be interpreted as indicating a rudimentary division, but, on the other hand, should be considered as due to an autogamous fusion of two nuclei. It is interesting to note that Geitler's interpretation of Karsten's observation is fully borne out by the fact that the nucleus of the young auxospore which is formed through the fusion of the two gametic nuclei in *Cyclotella Meneghiniana* always shows two nucleoli for some time even after the formation of the two valves (Text-figs. 52–57 ; Pl. IX, Figs. 19 and 21).

It was mentioned earlier, that Schmidt (1930, pp. 459–61) criticised Persidky (1929) on the ground that none of the earlier workers who studied auxospore-formation in the Centrales saw any of the nuclear stages recorded by Persidsky, evidently meaning thereby that if these stages really occurred during auxospore-formation, they would not have escaped the notice of the earlier workers. The following are very probably the reasons why the earlier workers failed to observe these nuclear changes. In the case of the Pennales auxospore-formation was known to be a sexual process brought about through the fusion of two gametic protoplasts (*cf.* Pfitzer, 1871 ; Klebahn, 1896 : Karsten, 1896, 1897 a, 1897b, 1899, 1900). In the Centrales on the other hand, no such fusion of two protoplasts is noticeable during auxospore-formation and hence this process was considered to be only a vegetative or an asexual process brought about by the mere enlargement of the contents of a vegetative cell. Again, it is only after the protoplast enlarges and begins to emerge out of the valves that the auxospore-formation becomes noticeable. And, by the time the auxospore becomes enlarged and noticeable, all the nuclear changes are already over and the enlarged auxospore shows only one functioning nucleus (the fusion nucleus). As Persidsky (1935, p. 129) states the nuclear changes should be looked for in the small auxospore-mother-cells, which are
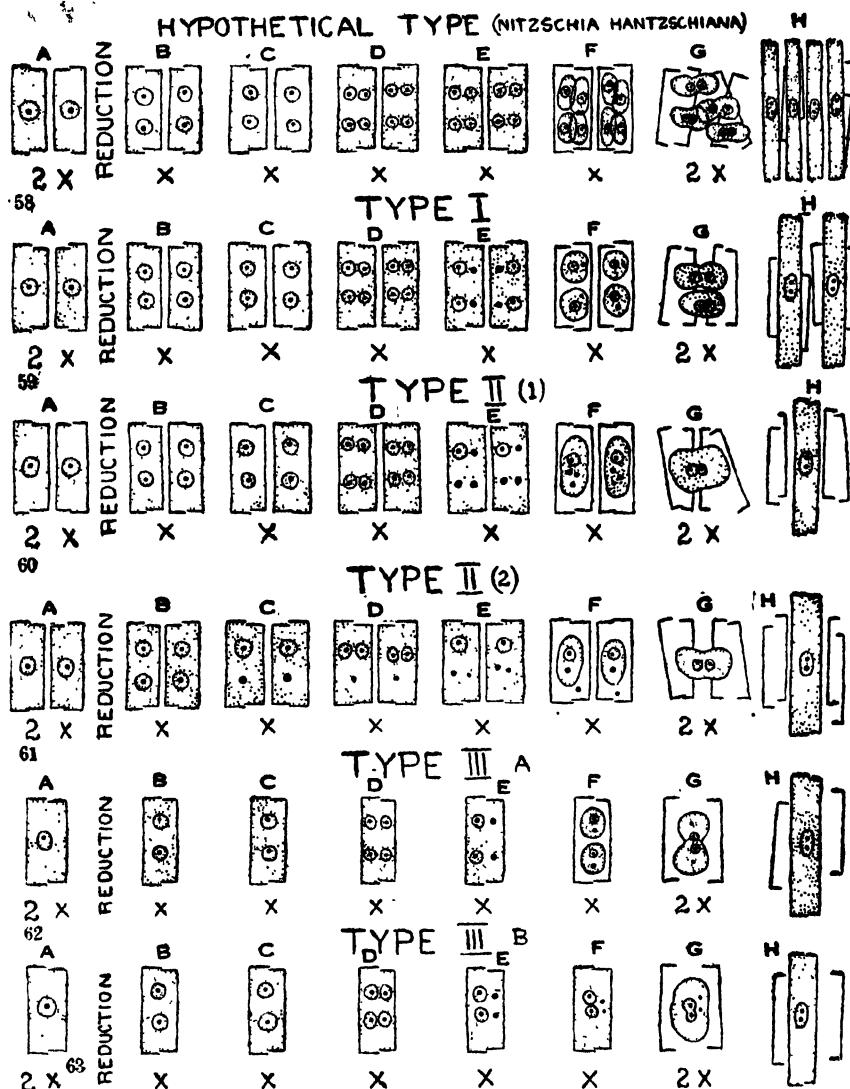
Text-figs. 58–63. *Cyclotella Meneghiniana* Kütz.—*Diagrammatic representation of various types of Auxospore-formation in Diatoms.*— Fig. 58. Hypothetical type (*Nitzschia Hantzschiana*). Two pairing cells present; all the four nuclei after reduction division functional : four zygotes formed. Fig. 59. Type I. Two pairing cells present ; only two nuclei in each cell functional after reduction division ; two zygotes formed. Fig. 60. Type II (1). Two pairing cells present. Only one nucleus in each cell functional after reduction division. Only one zygote formed. Fig. 61. Type II (2). Two pairing cells present. One nucleus extruded out from the cytoplasm after first division of meiosis and of the two nuclei formed in the second

division, one is functional and the other degenerates. Only one zygote formed. Fig. 62. Type III. *a.* No pairing cell present. Auxospore formed from a single cell. Two nuclei functional. The two gametes formed in the same cell fusing and forming one zygote. Fig. 63. Type III, *b.* No pairing cell present. Auxospore formed from a single cell. Division of the cytoplasm suppressed. Of the four nuclei that are formed after reduction division, two degenerate and the remaining two fuse (autogamy) : one zygote formed.

hardly distinguishable in external appearance from some of the smallest vegetative cells of the diatom. This the earlier authors evidently failed to do and hence their failure to observe these nuclear changes.

The only person among the earlier workers who came very near to finding out these nuclear stages was Karsten. He (Karsten, 1897*b*) noticed that the nucleus in the young auxospores of *Melosira nummuloides* and *M. moniliformis* had two nucleoli unlike the nucleus of the ordinary vegetative cell which showed only one nucleolus. But, he failed to recognise its real significance, *viz.,* that it is the fusion nucleus in the young auxospore, and so interpreted the phenomenon as a case of suppressed nuclear division.

Four types of auxospore-formation are known in the Diatomaceæ.[3]

In the first type (Text-fig. 59), two individuals come to lie near each other during auxospore-formation (Text-fig. 59*a*). The nucleus in each of the two cells divides twice and forms four nuclei (Text-fig. 59 *d*). The first division is meiotic (Text-fig. 59 *b*). Of the four nuclei that are formed two degenerate (Text-fig. 59 *e*). The protoplast of each cell then divides into two (Text-fig. 59*f*) and each daughter protoplast receives one functioning and one degenerating nucleus. The daughter protoplasts (gametes) of the two cells fuse and form two zygotes (auxospores), which then increase in size, develop new valves and form two new diatom cells (Text-fig. 59 *g* and *h*). This type of auxospore-formation is seen in *Rhopalodia gibba* (Klebahn, 1896), *Navicula viridula* (Karsten, 1896, 1899), *Cymbella lanceolata* (Geitler, 1927*a*) and *Anomœoneis sculpta* (Cholnoky, 1928), etc.

In the second type (Text-figs. 60 and 61), the protoplast does not divide and so only one gamete is formed in each cell. Two methods of auxospore-formation are seen in this type. In one method (*Surirella splendida,* Karsten, 1900) the nucleus of the cell divides twice and forms four nuclei (Text-fig. 60 *d*). Of these two divisions the first is meiotic. Of the four nuclei that are formed three degenerate while the fourth remains functional and forms the nucleus of the single gamete which is organised in the cell (Text-fig. 60 *e* and *f*). The gametes of the two cells fuse and form a single zygote (auxospore) (Text-fig. 60 *g* and *h*).

In the other method (Text-fig. 61), *e.g.,* *Cocconeis placentula* var. *klinoraphis,* *C. placentula* var. *pseudolineata* (Geitler, 1927 *b*) and

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[3] A good account of the different methods of auxospore-formation recorded among diatoms is given by Geitler (1932, 1935). The account given here is based largely on Geitler's account and the nomenclature of the types correspond to those of Geitler.

3

Navicula seminulum (Geitler, 1932), in each cell one of the two nuclei that is formed in the first division is extruded out with a small quantity of cytoplasm (" Richtungskörper ") (Text-fig. 61 *b* and *f*). The other nucleus divides again and, out of the two nuclei that are formed, one degenerates while the other forms the nucleus of the single gamete which is organised in the cell (Text-fig. 61 *d* and *f*). The two gametes fuse and form a single zygote (auxospore) (Text-fig. 61 *g* and *h*).

In type III (Text-figs. 62 and 63) unlike in types I and II there is no pairing of two separate cells. Two methods of auxospore-formation have been recorded in this type (type III A and type III B). In type III A the contents of a single cell divide into two gametes and the two gametes fuse with each other and form a zygote. In *Synedra ulna* which usually conforms to type I and forms in each cell two gametes which fuse with two other gametes formed in the opposite cell, Geitler (1939) recorded an exceptional instance in which the two gametes formed in a single cell fused with each other and formed a zygote (Text-fig. 62). In *Achnanthes subsessilis* Karsten (1899) found that the protoplast of a single cell divided into two uninucleate portions. In the later stages only one protoplast was seen by him with two nuclei and double the number of chromatophores. He presumes that the two protoplasts have fused. The actual fusion, however, was not observed by him.

In type III B (Text-fig. 63) there is no division of the protoplast but still a single auxospore is formed in each individual. This method is seen in *Amphora Normani* (Geitler, 1928*b*, 1932). In this case the nucleus divides and the resulting two nuclei fuse within the enlarging auxospore. The chromatophore, however, was observed to divide and this division of the chromatophore, according to Geitler, represents a division which has been suppressed. Geitler believes that a second nuclear division also takes place but has been overlooked. This method is a definite case of autogamy. Geitler (1932) includes *Libellus constrictus* (Karsten, 1896) and *Synedra affinis* (Karsten, 1897 *a*) under this method. He includes under this type, the following two centric diatoms also, *viz.*, *Chætoceros boreale* and *Ch. densum* (Persidsky, 1929).

In the fourth type auxospore-formation takes place without sexual fusion (*e.g.*, *Cocconeis placentula* var. *lineata* Geitler, 1927*b*). *Melosira* and other Centrales come under this according to Geitler (1932, p. 213).

Another interesting type may be mentioned in this connection. Pascher (1932, pp. 708–09, fig. 4) found that in *Nitzschia Hantzschiana* the contents of a cell divided into four daughter protoplasts. But no fusion was observed. Nothing is known regarding the nuclear changes connected with the formation of these protoplasts. Since four nuclei generally arise through two meiotic divisions, it is very probable that four nuclei are formed in this case also and each of the four daughter protoplasts receives one of the four haploid nuclei. The four daughter protoplasts very probably represent four gametes. This may, therefore, be considered a case where all the four nuclei remain functional and so four gametes are formed (Text-fig. 58).

A general survey of the different types of auxospore-formation among the diatoms shows that there has been a gradual diminution of sexuality within the group. *Nitzschia Hantzschiana* may be considered to be the most primitive condition. Here all the four haploid nuclei are presumably functional and four gametes are formed in each cell. Type I may be easily derived from a case like *Nitzschia Hantzschiana* through the degeneration of two of the four haploid nuclei that are formed. Here only two gametes are formed in each of the pairing cells.

In type II (Text-figs. 60 and 61) there is a further reduction of the sexual process. Here only one haploid nucleus finally remains functional and consequently only one gamete is organised in each pairing cell. These two gametes fuse and form only one zygote (auxospore).

In type III the sexual process is reduced still further. Here there is no pairing cell and fusion takes place between the products of the same cell. Two methods are seen in this type. In the first method (Text-fig. 62, type III A) two gametes which are formed in a single cell (in the same manner as in type I through the degeneration of two haploid nuclei) fuse with each other and form a single zygote (auxospore). This is a case of *automixis*. In the second method (Text-fig. 63, type III B) the sexual process is still further reduced. Here even the division of the protoplast of the single cell is suppressed, but the nucleus divides twice and forms four nuclei. The first division is presumed to be reductional. Of the four nuclei that are formed, two degenerate and the other two fuse and form a zygote nucleus and a single auxospore is formed. This is a case of *autogamy*.

In type IV sexuality was presumed to be completely absent and that auxospores were formed without sexual fusion of any kind.[4]

Thus we see within the group a gradual decrease in sexuality from a typical normal case as in *Nitzschia Hantzschiana* where all the four haploid nuclei are functional with the result that four gametes are formed to an extremely reduced method of sexual fusion (autogamy) as seen in type III B.

The centric diatoms were until now presumed to come under type IV where sexuality is completely absent and that auxospores in these forms were formed without sexual fusion of any kind. But auxospore formation in the few centric diatoms which have been recently investigated, viz., *Chœtoceros boreale*, *Ch. densum*, *Melosira arenaria*, *M. varians*, *Ditylum Brightwellii* and *Cyclotella Meneghiniana* is definitely the result of a sexual process (autogamy) and, therefore, comes under type III B and not under type IV, where auxospores are formed without sexual fusion of any kind as originally believed.

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[4] Geitler (1932, p. 213) includes the centric diatoms *Chœtoceros boreale* and *Ch. densum* under type III B, while *Melosira* and other Centrales are included by him under type IV. Recent investigations of Persidsky (1935) and Cholnoky (1933 *b*) have shown that auxospore-formation in *Melosira* also is a sexual process. Therefore Geitler in a later paper (1935) states that excepting in *Melosira*, sexual reproduction among the Centrales is not understood.

It is very interesting that all the few Centrales so far investigated show a sexual reproduction of such an extremely reduced type as autogamy. Further investigation of more members of the Centrales will probably show whether the less reduced types of sexual reproduction seen in the Pennales, *viz.*, types I, II and III A, are also found in the Centrales. In case the other types of reproduction should prove to be absent among the Centrales, then the Centrales should be considered to be a more highly evolved group than the Pennales which still show several less reduced types of sexual reproduction in addition to a few rare cases of autogamy.

In conclusion, the results of the present investigation may be summed up briefly as follows. The vegetative phase in *Cyclotella Meneghiniana* is definitely diploid as in the Pennales. and auxospore-formation is clearly the result of a sexual process as in the Pennales, though the sexual process is of a highly reduced type (autogamy). These observations on *Cyclotella Meneghiniana* are in full agreement with those of Persidsky (1935) made on *Melosira varians* and of Cholnoky (1933*b*) on *M. arenaria*. But Persidsky was not able to observe all the stages of the heterotypic division in *M. varians*. The observations of the other authors are still more meagre. In the present diatom, however, almost all the characteristic stages of the heterotypic division have been observed. The facts brought out in the present and other recent investigations already mentioned would appear to suggest that there is not much fundamental difference between the Pennales and the Centrales. It is very desirable that more members of the Centrales should be investigated in detail as regards auxospore-formation.

SUMMARY

The life-history and cytology of *Cyclotella Meneghiniana* Kutz. a centric diatom, as studied from living and fixed material are described in detail.

Cell-division takes place in the normal manner. The number of chromosomes observed in somatic mitosis appears to be above 60 (2 n).

During auxospore-formation the nucleus divides into four nuclei by two successive divisions, of which the first is a reduction division. Almost all the stages of the reduction division and the fusion of the two gametic nuclei have been followed. The haploid number of the somes appears to be 32–34 (n). Out of the four resulting nuclei two fuse and form the nucleus of the auxospore ; the remaining two nuclei degenerate.

It is suggested that the Centrales are not fundamentally different from the Pennales. Here also the auxospore-formation is the result of a sexual process as in the Pennales, only the sexual process in the Centrales is of an extremely reduced type, being completely autogamous. And the vegetative phase is diploid, the haploid phase in the life-history being represented by the four gametic nuclei.

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EXPLANATION OF PLATES

PLATE VIII

Fig. 1. Group of cells from the culture a few days after auxospore-formation; the very large cells are the newly formed cells from the auxospores and the very small cells are the future auxospore-mother-cells. × 80.

Fig. 2. Early stage in auxospore-formation. Contents emerging out of the valves. Note the covering membrane, the perizonium. × 700.

Fig. 3. Auxospore fully enlarged. × 700.

Fig. 4. Auxospore showing formation of the first valve, the epitheca. Note the valve is arched. × 700.

Fig. 5. Synizesis. × 1100.

Fig. 6. Pachytene, seen in valve view. × 1100.

Fig. 7. Pachytene. × 1100.

Figs. 8, 9. Diakinesis in two different foci. × 1100.

Fig. 10. Diakinesis in another cell. × 1100.

Fig. 11. First division metaphase. × 1100.

PLATE IX

Fig. 12. First division anaphase. × 1540.

Fig. 13. Second division early anaphase. × 1540.

Fig. 14. Second division anaphase slightly later than Fig. 15. × 1540.

Fig. 15. Second division metaphase in polar view. The other nucleus is out of focus and has not yet divided. Same as Text-fig. 46. × 1540.

Fig. 16. Second division anaphase (left) in one nucleus ; the other nucleus not yet begun to divide. Same as Text-fig. 47. × 1540.

Fig. 17. Four-nucleate stage ; two nuclei healthy and two degenerating (seen as dark bodies). Same as Text-fig. 50. × 1260.

Fig. 18. Fusion of the two functional gametic nuclei. Note the two degenerating nuclei seen as dark bodies. Same as Text-fig. 51. × 980.

Figs. 19–21. Protoplast with the fusion nucleus and two degenerating nuclei ; note the two nucleoli in the fusion nucleus. Fig. 19, just after fusion. Same as Text-figs. 52, 54 and 53. Fig. 19. × 1540 ; Figs. 20 and 21, × 1260.

Fig. 22. Protoplast with fusion nucleus (showing two nucleoli) ; the single degenerating nucleus present is out of focus. Same as Text-fig. 55. × 980.
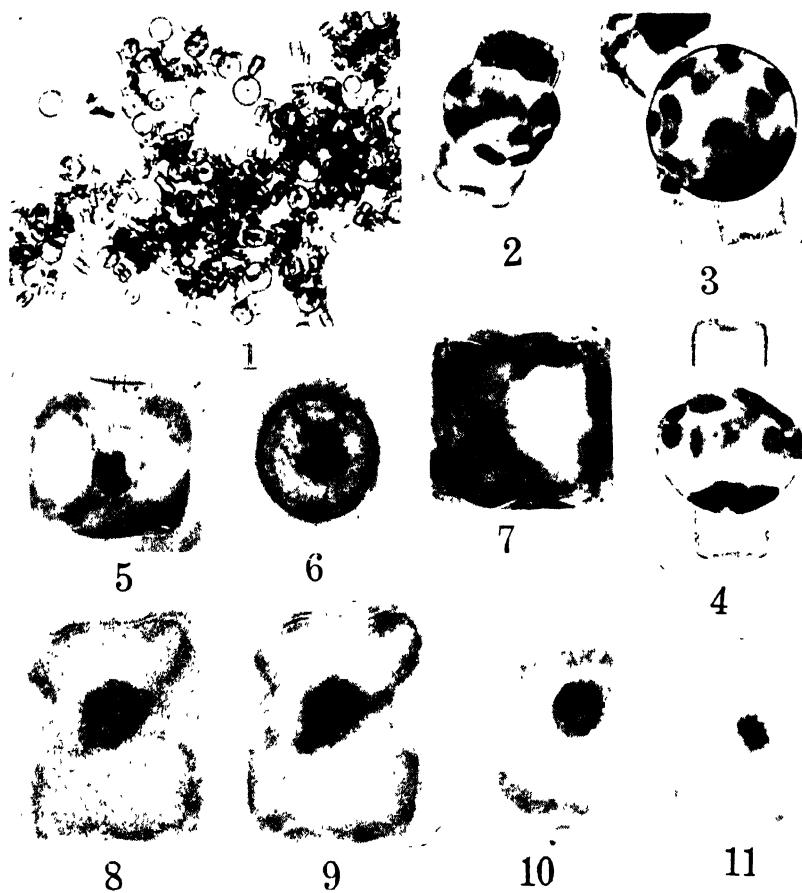
M. O. P. IYENGAR AND R. SUBRAHMANYAN

CYCLOTELLA MENEGHINIANA

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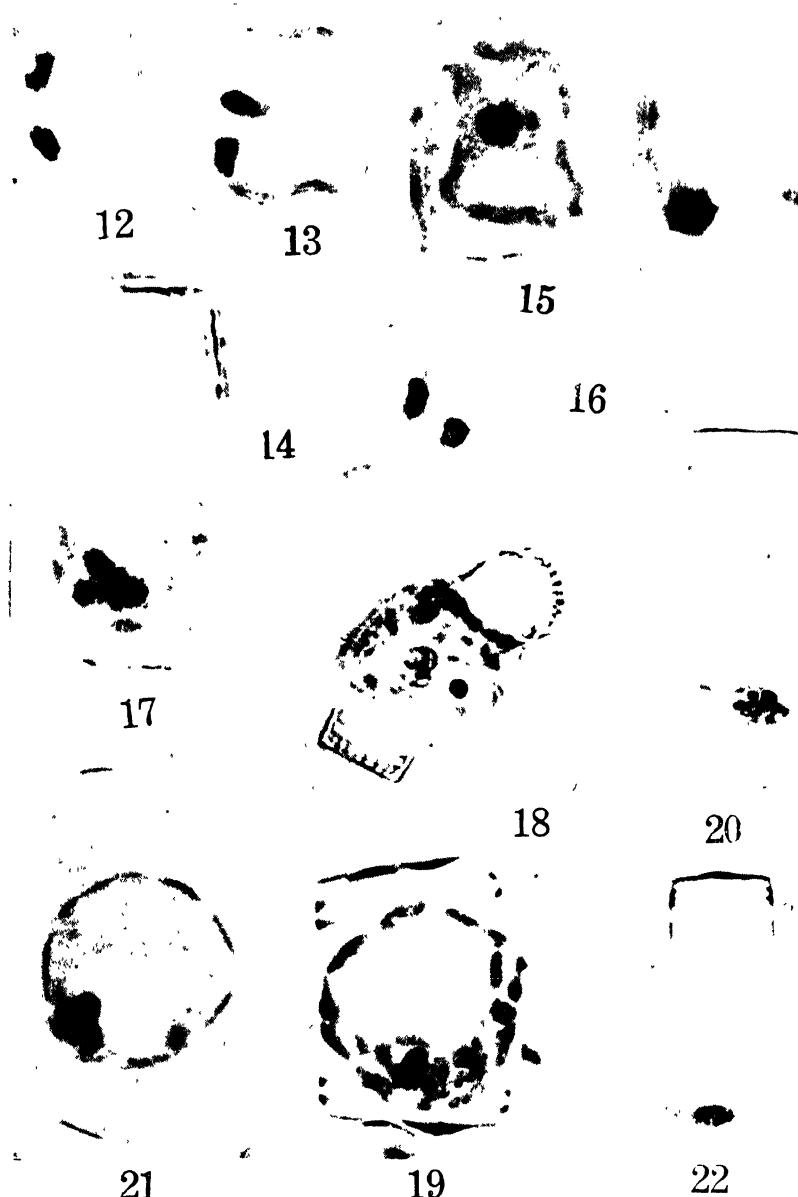
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CYCLOTELLA MENEGHINIANA

IMPORTANCE OF ANATOMY IN SYSTEMATICS OF POLYPORACEÆ

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IN the course of my continuing systematic study of Bengal Polyporaceæ, I have found the following anatomical characters to be of additional help in discrimination of species besides the characters of basidia and spores. Species are grouped under each distinctive character with text-figures (free-hand drawings from hand sections) in some cases.

1. (a) ENCRUSTED CYSTIDIA

1. *Polyporus zonalis* Berk.
2. *P. violaceo-cinerescens* Petch.
3. *Polystictus elongatus* Berk.
4. *P. abietinus* (Dicks.) Fries.
5. *P. personatus* B. & Br. (Fig. 1).
6. *Lenzites striata* Swartz.
7. *L. adustus* Massee.
8. *L. subferruginea* Berk.

1. (b) SIMPLE CYSTIDIA (NOT ENCRUSTED)

1. *Polyporus agariceus* Berk.
2. *Trametes floccosus* Bres.

1. (c) ENCRUSTED HYPHÆ

1. *Polyporus cervino-gilvus* Jungh.
2. *Trametes versatilis* Berk. (Fig. 2).

2. (a) SETÆ IN THE HYMENIAL LAYER

1. *Polyporus gilvus* Schwein.
2. *P. gilvus* forma *gilvoides* (Schw.) Fr.
3. *P. gilvus* forma *licnoides* (Mont.) Lloyd.
4. *P. cuticularis* (Bull.) Fries.
5. *P. calcuttensis* Bose.
6. *P. hookeri* Lloyd.
7. *P. radiatus* (Schw.) Fr.
8. *Polystictus cichoriaceus* Berk.
9. *P. tabacinus* Mont.
10. *P. xeranticus* Berk.
11. *Fomes conchatus* (Pers.) Fries. (Setæ bulbuos at the base) (Fig. 3 a).
12. *F. pachyphlæus* Patouill.
13. *F. lamaænsis* (Murr.) Sacc. & Trott.

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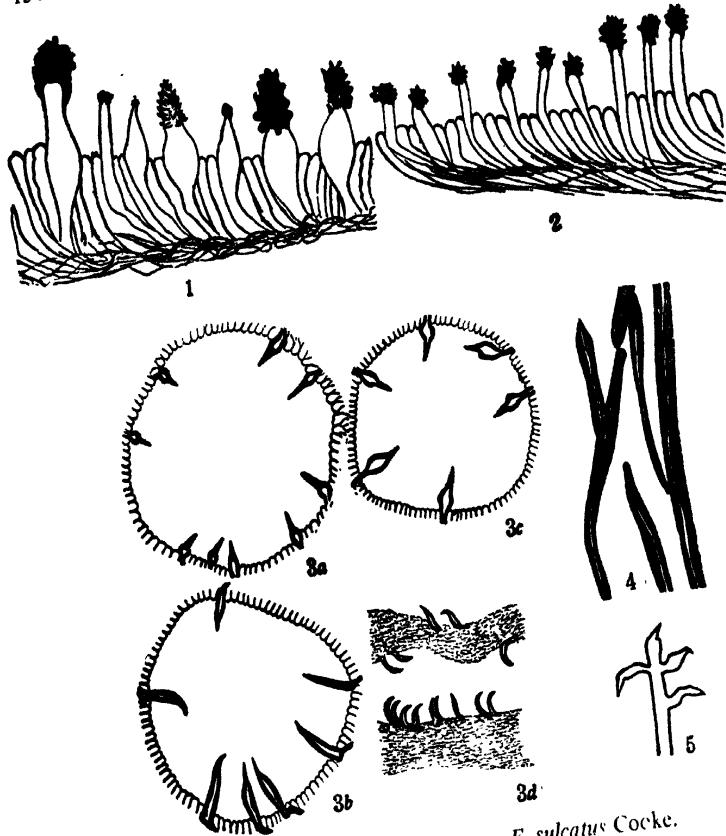
14. F. *hornodermus* (Mont.) Cooke. = F. *sulcatus* Cocke.
15. F. *senex* Nees & Mont. (Fig. 3 b).
16. F. *setulosus* Lloyd. (Few short setæ in new growths).
17. F. *caryophylli* (Rae.) Bres. (Subulate and ventricose setæ,
18. F. *igniarius* (L.) Fr. (Fig. 3 c) tubes becoming whitish with deposits of lime with age).
19. *Polyporus circinatus* Fries. (Fig. 3 d) (Setæ mostly curved at the apex).

2. (b) No SETÆ IN THE HYMENIAL LAYER.

1. *Fomes durissimus* Lloyd.
2. F. *fastuosus* Lév.
3. F. *rimosus* Berk.
4. F, *pectinatus* Klotzsch.
5. F. *pinicola* Fr.

6. *F. melanoporus* Mont.
7. *F. fomentarius* (L.) Fr.

2. (c) SETÆ EMBEDDED IN THE TRAMA

1. *Polyporus calcuttensis* Bose.
2. *Fomes pachyphlæus* Patouill. (Fig. 4).
3. *F. lamaœnsis* (Murr.) Sacc. & Trott.

2. (d) PRESENCE OF CURVED SETÆ ON THE UPPER SURFACT OF THE PILEUS

1. *Polyporus cuticularus* (Bull.) Fries.
2. *P. calcuttensis* Bose (Fig. 5).

3. A HYALINE CELLULAR INTERUPPTED LAYER ON THE UPPER SURFACE

1. *Fomes senex* Nees & Mont.
2. *Polyporus gilvus* forma *licnoides* (Mont.) Lloyd.
3. *P. gilvus* forma *gilvoides* (Schw.) Fr.
4. *P. gilvus* Schwein.
5. *Fomes fastuosus* Lév.
6. *F. pectinatus* Klotz.
7. *F. merrilli* (Murr.) Sacc. et Trott. (Fig. 6).
8. *Polyporus hookeri* Lloyd.
9. *Fomes durissimus* Lloyd.
10. *Favolus scaber* Berk. & Broome.
11. *F. brasiliensis* Fr.

4. RESINOUS PALISADE-LIKE TISSUE ON THE UPPER SURFACE

*1. *Ganoderma lucidus* (Leyss.) Fr. (Fig. 7).
2. *Amauroderma rugosus* Nees.
3. *Fomes subresinus* Murril.
4. *Ganoderma colossus* (Fr.) Bres.

5. NO PALISADE-LIKE TISSUE ON THE UPPER SURFACE, WHICH IS NON-RESINOUS

1. *Ganoderma applanatum* (Pers.) Pat.
2. *Fomes (Ganoderma) leucophæus* Mont.

By rubbing the upper surface with alcohol it can be seen that *Ganoderma lucidus* and *G. colossus* present a sticky and shining upper surface while *G. applanatus* and *Amauroderma rugosus* and *F. leucophæus* will show a non-sticky and dull (*i e.*, non-resinous) upper surface.

In *Ganoderma lucidus* when spore-discharge is very brisk, the colour of the hymenial surface is ash gray, then it turns whitish, and when the spore-discharge stops the colour becomes brownish.
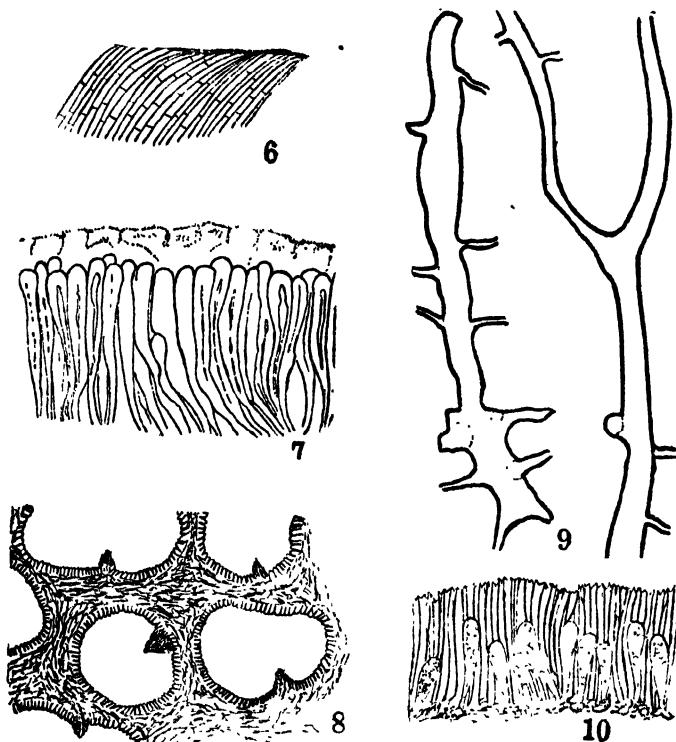
6. INDENTED OR LACERATED MARGINS OF THE GILLS AND
PORE-MOUTHS

1. *Lenzites striata* Swartz.
2. *Favolus brasiliensis* Fr.
3. *F. scaber* Berk. & Broome.

7. HYPHAL PEGS (CLUSTERS OF CLOSELY AGGLUTINATED HYPHÆ

IN THE FORM OF PROJECTIONS) INSIDE THE PORE-TUBES WHICH

NEVER BEAR BASIDIA ON THEM

1. *Polyporus thawaitesii* Berk.
2. *P. gramnocephalous* Berk. (pegs of low cone-form) (Fig. 8)
3. *Polystictus hirsutus* Fr. (We find majority of hill specimens of
P. hirsutus have greater number of hyphal pegs than those of
specimens collected from the plains.)
4. *P. sanguineus* (L.) Mey.
5. *P. versicolor* (L.) Fr.
6. *P. vinosus* (Berk.) Cooke.
7. *Favolus brasiliensis* Fr. (pegs of extremely low cone-form)
8. *Trametes serpens* Fr. (Poria)

9. *Hexagonia apiaria* Pers.
10. *H. discopoda* Pat. & Har.
11. *Dædalea unicolor* (Bull.) Fr.
12. *Polystictus zonatus* Fr.
13. *P. xeranticus* Berk.
14. *P. steinheilianus* Berk. & Lév. (pegs of cylindrical form).

N.B.—Some of the hill specimens of *Trametes lactinea* contain a
few hyphal pegs in their pore-tubes, while *T. lactinea*
collected from the plains does not usually show any
hyphal pegs in the pore-tubes.

8. ELONGATED THICK-WALLED CONDUCTING CELLS IN THE CONTEXT AND TRAMA

1. *Polyporus sulphureus* (Bull.) Fr. (Fig. 9).

9. THICK-WALLED AND DEAD FRINGE-HYPHÆ COVERING THE HYMENIUM

1. *Trametes lactinea* Berk. (in some cases).
*2. *Dædalea flavida* Lév. (fringe-hyphæ with bifurcated apices)
(Fig. 10).
3. *D. stereoides* Fr. (fringe-hyphæ with tapering apices).
4. *D. quercina* (L.) Pers. (fringe-hyphæ with bifurcated apices).
5. *Hexagonia discopoda* Pat. & Har.

In perennial species of Polyporaceæ growth takes place either
(I) from the living hyphal tissue *at the base* of the sporophore, com-
pletely covering the entire hymenial surface ; thus a stratified sporo-
phore is formed (as in many Fomes, some Polypores, etc.) ; or
(II) from the living hyphal tissue *at the margin* of the sporophore ;
thus an applante sporophore is produced showing the new zones added
year after year.

Specimens of Polyporaceæ can be distinguished from specimens of
Hydnum by bearing groups of basidia at the bases of pore-tubes in
longitudinal section, whereas in *Hydnum* species basidia are not found
between the bases of spines, such bases remaining distinct.

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MELANOPSAMMA RANJANII SP. NOV.: A NEW PARASITE OF SELAGINELLA*

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INTRODUCTION

THE fungi so far recorded on the various species of *Selaginella* have been listed by Gregor (1938) and by Mitra (1943). The specimen to be described here, differs from all the fungi mentioned in the above lists. It was found growing on living *Selaginella chrysocaulos* in a shaded place in the Lloyd Botanical Gardens, Darjeeling, in the month of September 1938. Even after a very careful search, however, not more than two infected plants could be found. But these infected plants showed the black perithecia of the fungus at the tip of almost every branch and spike, many of which had well-developed micro- and megasporangia (Fig. 1). These fructifications were not found on the branches near the base of the plant nor on other parts, such as stem and leaves. The only other external symptom of these plants was a little drooping of the infected branches. They were green and apparently were not killed by the parasite at the stage at which the material was collected. Unfortunately both the infected plants were preserved in formalin-acetic-alcohol, so that no cultural studies or inoculation experiments could be made. The writer had thus to be content with a study based on teasings and microtome sections of the original material.

OBSERVATIONS

Host-Parasite Relation

A longitudinal section through the infected tip (Fig. 1) revealed the very interesting feature that the hyphæ of the parasite were present only in the xylem of the vascular bundle. To ascertain the extent of penetration, transverse sections of the stem at various heights were examined. Sections of the stem at the very base did not show infection. The presence of the fungus was first detected in sections about 1·8 cm. below the lowest branch whose tip bore the perithecia. Above this portion every section up to the very top showed the presence of the parasite.

A careful examination of the transverse section of the infected part of the main stem or the branches (Figs. 3 and 4) shows the presence of hyphæ inside the xylem only, the phloem, pericycle, endodermis and cortex being quite free from the parasite. The hyphæ are found

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in quite large numbers in all parts of the xylem, including protoxylem and many of the tracheids are even found to be clogged (Fig. 4). Near the tip of the branches where the xylem breaks up into isolated strands separated by parenchyma, the hyphæ are found inside the xylem as well as the parenchymatic cells, but even here they do not grow into the pericycle or parts of the cortex. At the very tip of the branch, however, all the undifferentiated parenchymatic cells are attacked and this infected part gradually merges into a pseudo-parenchyma formed entirely of fungal hyphæ, on which the perithecia are situated (Fig. 1). The infected tracheids of the stem are continuous with those of the branch trace. The hyphæ also enter the xylem of the leaf trace but do not affect any other part of the leaf (Fig. 1). The growth of the hyphæ inside the xylem evidently interferes with the flow of water and this explains the drooping of the infected branches already referred to. A longitudinal section of the stem (Fig. 5) shows a luxuriant development of the hyphæ within the tracheids. They are fairly thick and frequently show branching, anastomoses and H-pieces.

Description of the Parasite

Except the perithecia no pycnidial or other imperfect conidial stages have been found.

Perithecia.—At the tips of vegetative shoots or sporangiferous spikes one can see minute globose, carbonous perithecia present superficially in groups of two to five, solitary ones being extremely rare. They are hard, smooth and devoid of hairs. These seem to be situated directly on the tips of branches but a longitudinal section through the infected region shows that they are seated on a small pseudoparenchymatous base which does not form a well-marked external stroma. This pseudoparenchymatic base, comes out with the perithecia if they are separated (Fig. 6 A). The venter of the perithecium is spherical (Fig. 2). There is no beak, but the ostiole is situated as a clearly defined pore in a minute round papilla at the top (Fig. 6 A). Inside the perithecium are asci and paraphyses originating from the basal region and the sides. They are absent in the region of the neck where they are replaced by periphyses. The size of the perithecia varies greatly and sometimes younger perithecia are found attached to the same pseudoparenchymatic base as the mature ones. The mature perithecia range from 209 μ to 383 μ in diameter. A perithecium of average size measures about 300 μ.

Asci.—The asci are cylindrical or club-shaped. They have got short, tapering stalks and possess slightly flattened bases for attachment (Fig. 6 B). Each ascus contains eight ascospores generally arranged in a single series (monostichous), but here and there some of the ascospores show a distichous arrangement (Fig. 6 B). The asci are hyaline to somewhat translucent and contain oil globules, which come out when teased. The asci are 80–88 μ long and 12–14 μ broad.

Paraphyses and Periphyses.—Paraphyses are free, persistent, unbranched, non-septate and do not anastomose with each other (Fig. 6 C). They are hyaline to translucent and contain oil globules. The paraphyses are shorter and narrower than the asci, but like them possess a
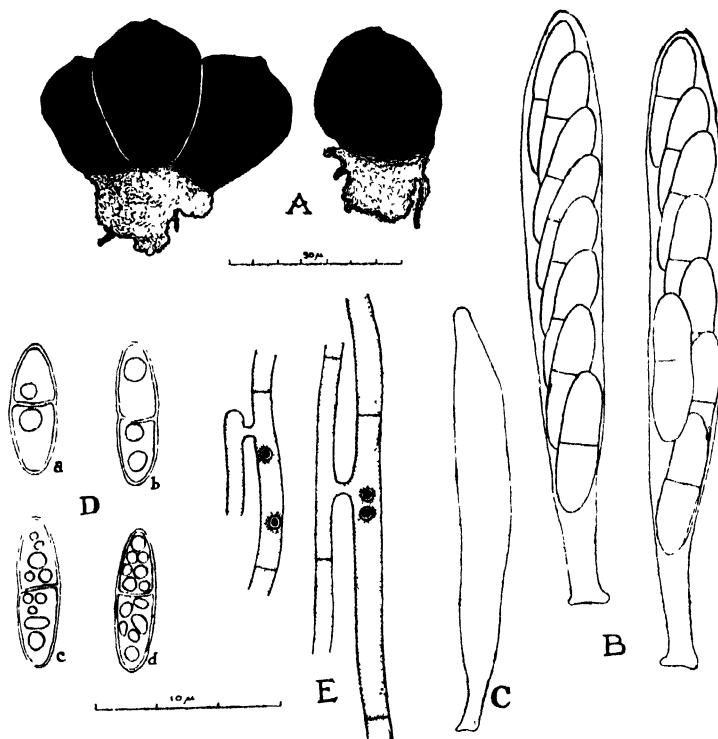
Fig. 6. *Melanopsamma Ranjanii* sp. nov.—Camera lucida drawings of peri-thecia, asci, etc. Fig. A, Perithecia with basal pseudoparenchymatic mass generally found in groups but in rare cases single. ⟨ 64. Fig. B, Asci with monostichous ascospores. × 800. Fig. C. Paraphysis. ⟨ 800. Fig. D, Bi-celled ascospores showing stages in maturation and increase of guttulæ in older spores. × 800. Fig. E, Binucleate hyphæ and H-pieces from L.S. of stem in xylem region. × 800.

tapering stalk, a flattened base and a rounded apex. They measure on an average $72\,\mu \times 10 \cdot 5\,\mu$. The periphyses never come out of the ostiole.

Ascospores.—The ascospores are ovoid to spindle-shaped, sometimes slightly curved, hyaline and bicelled. The equatorial septum divides the spore into two almost equal halves with a very slight constriction in the middle, which may sometimes be absent. Their ends are gra-dually rounded although in a few cases they are more pointed than usual. At first each cell of the ascospore is uni-guttulate but as it ages the guttulæ increase in number, so that the cells of the mature spore become multiguttulate (Fig. 6 D). This makes the septum difficult to see without proper staining. The ascospores vary from 20 to $28\,\mu$ in length and 7 to $8\,\mu$ in breadth. The average size of the ascospore is $26\,\mu \times 7\,\mu$.

Mycelium.—The loose hyphæ inside the tracheids are branched and consist of elongated cells which are 30–$60\,\mu$ long and $2 \cdot 5$–$5\,\mu$ broad.

They frequently show anastomoses and H-pieces (Fig. 5). These cells contain a number of vacuoles. The cells near the pseudoparenchymatic base are rectangular to iso-diametric.

Cytology.—The mycelium, which shows fusions and H-pieces at various places, shows two nuclei in each cell. These nuclei may lie very close to each other or may be more or less apart (Fig. 6 E). They measure about 2 μ in diameter and consist of a deeply staining central body with a white halo around it. In a few cases very much elongated nuclei were found. These were probably in the course of division. Youngest perithecia are uniformly pseudo-parenchymatous with outer layers of thicker cells. In slightly older ones the centre is occupied by a mass of deeply staining hyphæ, the cells of which are bi-nucleate. The nuclei in this case were much smaller. No definitive nucleus or other stages in the cytology of the ascus were observed. Each cell of the ascospore contains a single nucleus.

Identity of the Fungus.—The superficial, glabrous and unbeaked nature of the carbonous perithecia, which are not situated on a distinct stroma, together with the presence of hyaline, ovoid, bi-cellular spores indicate that this fungus belongs to the genus *Melanopsamma* Niessel (Fam. Sphæriaceæ). The fungus also agrees with the description given for *Melanopsamma* by Saccardo (1887) and by Winter (1887). A large number of species of *Melanopsamma* is known and many of them grow on rather primitive phanerogams, such as the Archichlamydeæ, Gymnosperms, etc. But none has been recorded so far on *Selaginella*. The habitat of the fungus together with the measurements of perithecia, asci, ascospores, etc., show that it is a new species. I have much pleasure in naming it *Melanopsamma Ranjanii* sp. nov. after Dr. Shri Ranjan, Professor of Botany, University of Allahabad. It may be noted here that this is also the first record of *Melanopsamma* on any host in India (*cf.* preliminary note by Mitra, 1943). Neither Butler and Bisby (1932) nor Mundkur (1938) mention anything about this genus. This is rather surprising as species of *Melanopsamma* are well represented in the tropics—a large number having been recorded from the Philippines. Gwynne-Vaughan and Barnes (1922, p. 153) also remark in connection with the Sphaeriales that "there is no doubt that a study of the tropical forms at present very incompletely known, will greatly increase their number."

DISCUSSION

A great deal of difference of opinion exists as to the limits of the Sphæriales. Petrak (1924) has shown that many species till then regarded as simple Sphæriales really belonged to the Pseudosphæriales, which are related to the Dothideales as their perithecia are in reality unilocular stroma. Many modern writers (Miller, 1928; Theissen and Sydow, 1918) have recognised this differentiation and have given certain criteria for distinguishing between a simple perithecium and an unilocular stroma. According to Miller (1928) the attempt by Petrak (1924) and others to separate these two on the basis of thickness of wall is not fruitful. On the other hand he considers the presence of a true perithecial wall to be a fundamental criterion of a true perithecium,

Chesters (1938) agrees with this. Presence of a true perithecial wall is correlated with other characters. The centre of the perithecium is not pseudoparenchymatous, asci do not ripen in series so that ripe and unripe asci are not found together, and true paraphyses and periphyses occur. If we apply the above criteria we see that *Melanopsamma Ranjanii* possesses true perithecia and so belongs to the Sphæriales. Chesters (1938) in his studies on two species of *Melanomma* came to the conclusion that these two species ought to be placed in the Pseudosphæriales, and opines that "It is probable that species of *Bertia* and *Melanopsamma* will be found to have a similar development to that of *Melanomma* and to belong to the Pseudosphæriales." The present study shows that Chesters' (1938) prediction is not true for all the species of *Melanopsamma*.

Diagnosis of Melanopsamma Ranjanii *sp. nov.*

Përithecia superficialia, in summitate ramorum, gregaria, 2–5 simul, spherica, carbonacea, lævia, ostiolum in minuta papilla, 209–383 μ diam. ; asci cylindrici vel clavati, octo-spori, hyalini, 80–88 ⩗ 12–14 μ ; paraphyses clavatæ ; sporidia monosticha, ovoida vel fusoidea, apice rotundo, hyalina, uniseptata, multi-guttulata dum matura, 20–28 ⩗ 7–8 μ ; mycelia in xylemo caulis hospitis tantum, sed inficientia alias parenchymaticas cellas summitatis, bi-nucleata, cum frequentes anastomoses et H-partes.

*Hab.*—In summitate ramorum et spicis *Selaginellæ chrysocaulis*, Darjeeling, India, September 1938.

Perithecia superficial, at tips of branches, gregarious, in groups of 2–5, spherical, carbonous, smooth, ostiole in a minute papilla, 209–383 μ in diameter ; asci cylindrical or club-shaped, eight-spored, hyaline, 80–88 × 12–14 μ ; paraphyses clavate ; ascospores monostichous, ovoid to spindle-shaped, ends rounded, hyaline, uniseptate, multi-guttulate when mature, 20–28 × 7–8 μ ; mycelia only in xylem of the stem of the host but infects other parenchymatous cells at the tip, bi-nucleate, shows frequent anastomoses and H-pieces.

Hab.—At the tips of branches and spikes of *Selaginella chrysocaulos* in Darjeeling, India, September 1938.

Type specimen deposited in the Herbarium of the Botany Department, Universtiy of Allahabad, India.

SUMMARY

1. *Melanopsamma Ranjanii* sp. nov. is recorded as a new parasite of *Selaginella chrysocaulos*. This is also the first record of *Melanopsamma* on any host in India.

2. The black perithecia occur only at the tips of branches which do not show any other symptom except drooping.

3. In the stem of host, the parasite is present only in the xylem region without affecting other parts. Some tracheids are found to be totally clogged by the parasite. Here many hyphæ showing H-pieces and binucleate cells are found.
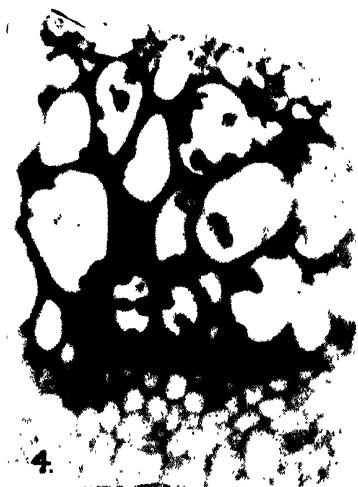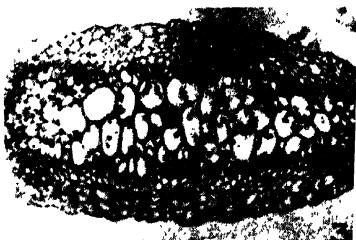
4. At the tip of branches the young parenchymatous cells are attacked and this infected part gradually merges into a pseudoparenchyma formed entirely of fungal hyphæ.

5. It has been shown that *Melanopsamma Ranjanii* possesses true perithecia and so belongs to the true Sphæriales.

6. A full description of the parasite is given.

In conclusion, the author wishes to express his thankfulness to Professor Shri Ranjan, D.Sc., Head of the Department of Botany and Dean of the Faculty of Science, University of Allahabad, for his valuable suggestions and for kindly going through the slides and manuscript. The author is also indebted to Father Jerome of St. Joseph's Seminary, Allahabad, for help in translating the diagnosis into Latin.

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EXPLANATION OF PLATE

Melanopsamma Ranjanii sp. nov. on *Selaginella chrysocaulos*

Fig. 1. L.S. through tip of an infected spike with mature sporangia of the host, showing terminal perithecia, affected conducting strand of stem and leaves.

Fig. 2. L.S. through a perithecium showing asci, paraphyses, wall and spherical venter.

Fig. 3. T.S. through the vascular bundle of the stem in the infected region showing hyphæ in xylem.

Fig. 4. Hyphæ in xylem. Note their absence in phloem and pericycle.

Fig. 5. L.S. of the stem showing anastomosing hyphæ in xylem.

DEVELOPMENT OF THE EMBRYO-SAC IN THE CONVOLVULACEÆ

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THERE are a number of contradictory statements in the literature on the embryology of the Convolvulaceæ, particularly with reference to the presence or absence of parietal cells. Peters (1908) investigated *Cuscuta europea* and *Convolvulus sepium* and reported that the primary archesporial cell in both of them cuts off a wall cell and the embryo-sac develops according to the normal type. Asplund (1920) also reported the formation of a primary wall cell in *Cuscuta lupuliformis*. Svensson (1925), on the other hand, holds the view that the parietal cells described in *Cuscuta* and *Convolvulus* by Peters and Asplund were probably derived from the epidermis and are not true parietal cells. Dahlgren (1927) shares the views of Svensson and states that parietal cells are definitely absent in *Cuscuta lupuliformis* and *C. epithymum*. Macpherson (1921) who studied the embryo-sac of *Cuscuta gronovii* and *Convolvulus sepium* could not observe the early stages of its development. Kenyan (1929) investigated *Ipomea trifida*. He found numerous archesporial cells, formation of the parietal cells and a normal type of embryo-sac. His observations also indicate that the inner cells of the integument are consumed during the growth of the embryo-sac. Mathur (1934) reported the occurrence of a definite primary parietal cell in *Convolvulus arvensis*. Johri (1934) found that in *Cuscuta reflexa* the hypodermal archesporial cell functions directly as the megaspore-mother cell and the wall cells are completely absent. He has further stated that the development of the embryo-sac conforms to the *Scilla*-type. Smith (1934) in several species of *Cuscuta* growing in North Carolina and Tiwary and Rao (1936) in *Evolvulus nummularis* found that the embryo-sac develops according to the normal type. They do not say anything about the parietal tissue. Raghava Rao (1940) in a recent paper describes the development of the embryo-sac in *Ipomea Learii, I. staphylina, I. hederacea, Argyreia speciosa* and *Evolvulus alsinoides*. He reports the formation of a primary wall cell in *Ipomea Learii* and its absence in *I. staphylina* and *Evolvulus alsinoides*. He says nothing about *Ipomea hederacea* and *Argyreia speciosa* in this connection.

The present investigation deals with the development and structure of the embryo-sac in *Jacquemontia violacea* Choisy, *Ipomea pulchella* Roth., *I. Horsfalliae* Hook. *f., I. obscura* Ker-Gawl., *I. sepiaria* Koenig and *Operculina Turpethum* Manso. Material of these was collected from the environs of Bombay and studied according to the customary methods.

OBSERVATIONS

Ovary and Ovule.—The ovary of the Convulvulaceæ is generally described as bilocular, with two axile ovules in each loculus arising from near the base as shown in Fig. 1. Such a description however is not true for all the stages. Serial transverse sections of young ovaries, as illustrated in Fig. 2 for *Jacquemontia violacea*, reveal that the ovule-bearing carpel margins do not meet in the centre. The ovary therefore is at first unilocular and the placentation marginal and parietal. The two placentas are separated near the base of the ovary by a narrow channel. Near the top of the ovary the margins of the carpels become even more clear and those of the opposite sides are seen to be quite free. The line of fusion between the two carpels is quite clear even in the style. Thus the microscopical examination of the young stages of the ovary shows that the union of the carpels is not so thorough as might appear from the outside. These stages also clearly bring out the bicarpellary nature of the gynoecium.

The ovule initials as usual arise as papilla-like outgrowths from the placenta (Fig. 3). Soon the terminal part of the initial curves so as to make an angle of 90 degrees with the basal part, which forms the funicle of the ovule. It is about this stage that the single integument arises from the base of the nucellus (Fig. 4). The bending of the ovule continues until the adult anatropous form is attained. The funicle is very short. The nucellus is very small as compared with the thick integument. The micropyle is long and extremely narrow as described by Kenyan (1929) in *Ipomea trifida*. This is clear from Figs. 5 and 7, which illustrate the structure of the ovules of *Jacquemontia violacea*.

Development of the Embryo-sac.—The archesporium is hypodermal and differentiates very early, even before the origin of the integument. In *Jacquemontia violacea* sometimes two or three archesporial cells are seen to occur side by side in the same nucellus (Fig. 6). In all other species in every case only one archesporial cell was noted.

The archesporial cell cuts off a parietal cell immediately after its formation (Figs. 3 and 16), which divides further to form a distinct, parietal tissue (Figs. 4, 5 and 17). The first division wall formed in the parietal cell is periclinal (Fig. 17) or anticlinal (Fig. 4). The second division may be either anticlinal or periclinal, and the later divisions occur irregularly. As the result of these divisions in the primary wall cell, the megaspore-mother cell is covered by about 4–7 layers of cells (Fig. 5). Raghava Rao (1940) states that the first division of the primary wall cell in *Ipomea Learii* is anticlinal and a similar behaviour has been observed by Mathur (1934) in *Convolvulus arvensis*. From the occurrence of primary wall cell in every one of the six species investigated during the course of the present work, it appears, in spite of Dahlgren's (1927) strong criticism, that Peters (1908) was correct in his assertion about the occurrence of parietal cells in the Convolvulaceæ investigated by him. Also, therefore, many of the observations on the Convolvulaceæ, where the presence of the parietal cells has been denied, appear to be doubtful and deserve reinvestigation,
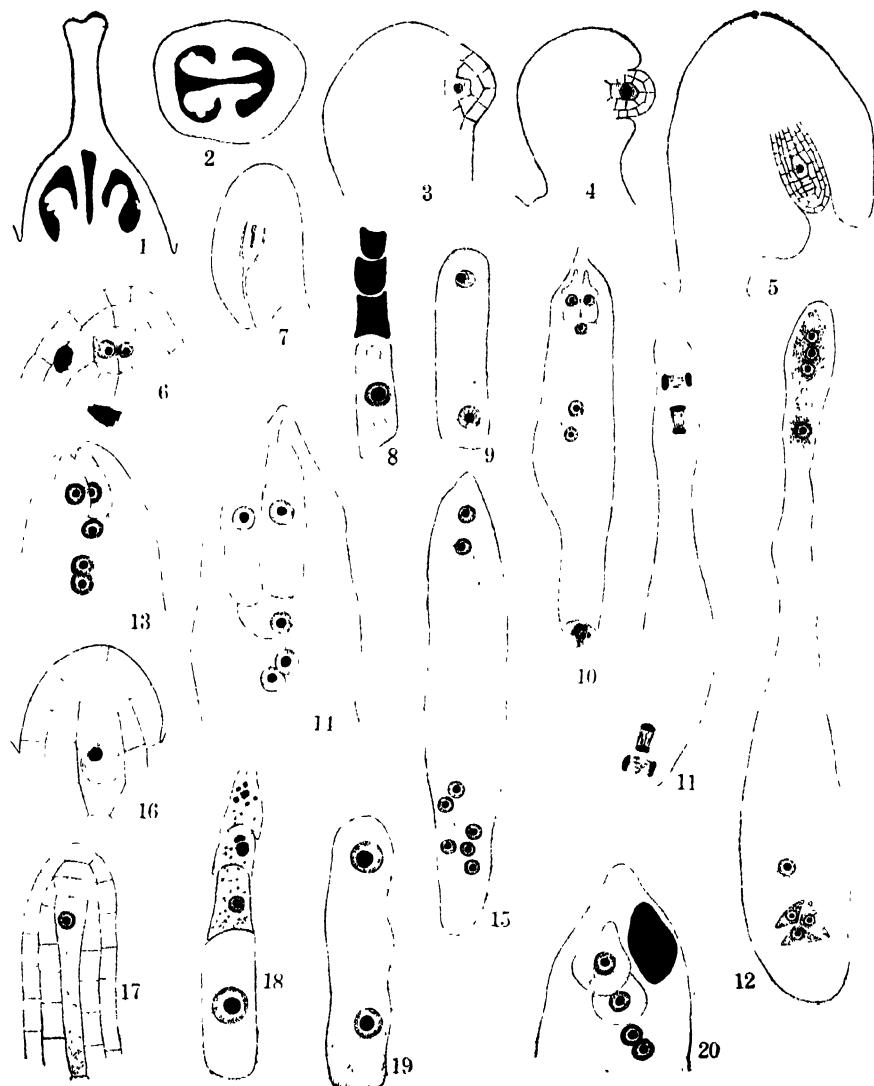
Figs. 1–20.—Figs. 1–10. *Jacquemontia violacea.*—Fig. 1. L.S. of a gynœcium with the ovules at the stage shown in Fig. 5. × 60. Fig. 2. T.S. of a young gynœcium slightly above the middle of the ovary. × 60. Fig. 3. A young ovule showing the formation of the primary wall cell. × 440. Figs. 4 and 5. Two ovules showing the megaspore-mother cell and the development of the nucellus. × 260. Fig. 6. An ovule showing two megaspore-mother cells. × 570. Fig. 7. An ovule at the tetrad stage showing the thick integument and the long narrow micropyle. × 260. Fig. 8. A linear tetrad of megaspores with the three micropylar megaspores degenerating. The chalazal one is developing into the embryo-sac. × 950. Fig 9. A 2-nucleate embryo-sac. × 950. Fig. 10. A mature embryo-sac. × 570. Figs. 11–13. *Ipomea pulchella.*—Figs. 11 and 12. Two stages in the development of the

8-nucleate embryo-sac. × 570. Fig. 13. Micropylar region of a mature embryo-sac, showing the egg-apparatus and the polar nuclei. × 260. Figs. 14–15. *Ipomea Horsfalliæ.*—Fig. 14. Micropylar portion of an embryo-sac showing the egg-apparatus and the polar nuclei. Fig. 15. An abnormal embryo-sac showing 6 nuclei at the chalazal end and 2 at the micropylar end. × 570. Figs. 16–20. *Operculina Turpethum.*—Fig. 16. An ovule showing the megaspore-mother cell and the formation of the primary wall cell. × 950. Fig. 17. An ovule showing a later stage in the development of the megaspore-mother cell. × 570. Fig. 18. A linear tetrad of megaspores. × 950. Fig. 19. A binucleate embryo-sac. × 950. Fig. 20. Micropylar portion of a mature embryo-sac with one of the synergids degenerating. × 440.

The megaspore-mother cell forms a linear tetrad of megaspores by two successive divisions,—*cf.* Fig. 8 for *Jacquemontia violacea* and Fig. 18 for *Operculina Turpethum.* Similar stages have been observed also in the other investigated species belonging to the genus *Ipomea.* The chalazal megaspore functions in every case and develops into the 8-nucleate embryo-sac according to the normal type (Figs. 9–10, 11–13 and 18–20). One or two prominent vacuoles can be discerned in the cytoplasm of the functional chalazal megaspore even before the first division of the nucleus (Figs. 8 and 18). In the 2-nucleate stage of the embryo-sac they are replaced by a prominent central vacuole (Figs. 9 and 19). The enlargement of the embryo-sac at first takes place at the expense of the surrounding nucellus cells. In *Jacquemontia,* most of the nucellus except the outermost layer is absorbed by the 2-nucleate stage. The integument at this stage is 12–15 cells thick. During the course of further development the inner layers of the integument are also absorbed. The 4-nucleate stage of the embryo-sac calls for no remarks. During the mitotic divisions preceding the 8-nucleate stage, of the two spindles at each end, one is placed parallel, the other at right angles to the long axis of the embryo-sac (Fig. 11).

The mature embryo-sac is long and narrow, its length being generally about six times the width (Fig. 16). In *Ipomea pulchella,* it is even longer, and the micropylar end is narrower than the chalazal (Fig. 13). This species is characterised by a very much smaller nucellus and comparatively thicker integument than the rest.

The synergids of *Operculina Turpethum* are nearly as long as broad (Fig. 20) ; of other species they are generally about three times as long as broad (Figs. 10, 13 and 14). The apex of the synergids except in *Operculina* is usually drawn out. Hooks of a small size have been observed on the synergids of *Jacquemontia violacea* (Fig. 10) and *Ipomea pulchella.*

Definite antipodal cells are always formed (Figs. 10 and 12). They are organized generally even before the cells of the egg-apparatus (Fig. 12), but are quite ephemeral and begin to degenerate as soon as they are formed.

The two polar nuclei travel towards the micropylar end of the embryo-sac and stay near the egg-apparatus (Figs. 13 and 20). A fusion of the polar nuclei has not been observed even in those embryo-sacs, where the egg-apparatus appears to be fully ripe. Their fusion,

as pointed out by Raghava Rao (1940), is perhaps delayed until the second male nucleus approaches them.

Macpherson (1921) stated that the cells of the nucellus are rich in starch. Dahlgren (1927) pointed out that these cells may really belong to the integument. The writer's observations as regards the distribution of starch agree with those of Dahlgren. Starch is present in the cells of the integument, but not in the nucellus.

An abnormal embryo-sac has been observed in *Ipomea Horsfalliæ* (Fig. 15). It shows six nuclei at the chalazal end and only two at the micropylar end. Probably one of the micropylar nuclei at the 4-nucleate stage of the embryo-sac was here pushed towards the chalazal end.

SUMMARY

Development of the embryo-sac has been studied in six species of Convolvulaceæ belonging to three genera, namely, *Jacquemontia violacea*, *Ipomea pulchella*, *I. Horsfalliæ*, *I. obscura*, *I. sepiaria* and *Operculina Turpethum*. Two or three primary archesporial cells are occasionally present in *Jacquemontia*. Otherwise there is always only one hypodermal archesporial cell, which differentiates much before the origin of the integument. Parietal tissue is formed in all the species, the archesporial cell cutting off a primary wall cell in every case. The development of the embryo-sac corresponds to the normal type. The antipodals are short-lived. The fusion of the polar nuclei is long delayed.

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REVIEW

Plant Viruses and Virus Diseases. By F. C. Bawden. Second entirely revised edition; Vol. XIII, 1943, Waltham Mass., U.S.A. Messrs. Chronica Botanica, Calcutta. Messrs. Macmillan & Co., Ltd. Pp. 294. 48 illustrations. Buckram, $ 4·75.

THE publication of this second edition of a quick-selling and important book on a rapidly changing subject is a very welcome enterprise on the part of the editor of the New Series of Plant Science Books, Dr. Frans Verdoorn. The loss of the type of the first edition during the invasion of the Netherlands in 1940, although to be deplored, has resulted in a complete revision of the first edition so as to include all the dynamic changes that have taken place in our 1939-conception of plant viruses. Frequent attempts to bring our knowledge up to date has to be made in this fascinating group of ultra-microscopic plant pathogens which is now the domain of the Pathologist cum Chemist. The opinion of the author that the number of stubborn orthodox biologists who still regard these specific plant virus nucleo-proteins as something other than the viruses themselves has dwindled down, is a tribute to the overwhelming mass of positive evidence that has accrued in the past ten years.

Although this edition has been completely revised, with a number of chapters rewritten, the general arrangement of the subject-matter remains essentially the same as in its predecessor. The chapter headings are : (1) Introductory Survey ; (2) and (3) Symptomatology (External and Internal) ; (4) Transmission ; (5) Relationships between viruses and their insect vectors ; (6) Virus strains, mutations, and acquired immunity ; (7) Serological reactions of plant viruses ; (8) Methods of purification ; (9) Properties of purified virus preparations ; (10) Optical properties of purified virus preparations ; (11) Inactivation of viruses ; (12) The sizes of virus particles ; (13) Physiology of virus diseased plants ; (14) The classification of viruses ; (15) The control of virus diseases ; (16) Discussion on the origin and multiplication of viruses. Bibliographies which are fairly exhaustive and commensurate with the subject-matter dealt with terminate chapters. There are 48 illustrations in all, thus showing an increase of eleven over the first edition.

From the economic point of view the revised chapter on " relations between viruses and their insect vectors," provides a very stimulating reading and in addition permits of visualizing the enormous and complicated problem that this mode of transmission offers in the field. Reverting to the academic problems of plant viruses Mr. Bawden has presented data in a very forceful and lucid way which might be called exact but not exacting. To the academically minded person, therefore, Chapters 7 to 12 will appeal as most convincing evidence of the chemical nature of these plant virus particles. One cannot, however, refrain from remarking that more constructive suggestions on the improvement of virus nomenclature has not found a place in this edition as well.

This, of course, is due to the absorbing interest that a virus pathologist is apt to take on the multifarious aspects of this science—chemical, entomological, serological, etc., that he often finds himself too engrossed to emerge out and tackle the nonetheless intricate and thought-provoking job of introducing the latinized binomial system of nomenclature. From the academic degree point of view in this country it is difficult to introduce virus pathology along with Mycology for the degree courses until the virus nomenclature is put *on a par* with its sister pathological subjects. Nevertheless, Mr. Bawden's book should find a place in the science libraries of every college, for it affords an excellent reading, to the student of general Botany, of a hitherto little known branch of Plant Pathology. To the more advanced Plant Pathologist and Virus Physiologist this neat volume is indispensable, for, it critically sums up all the latest knowledge of *in vitro* and *in vivo* reactions of plant viruses.

T. S. SADASIVAN.

THE EMBRYO-SAC OF *HECKERIA*
SUBPELTATA KUNTH.

BY B. G. L. SWAMY

Heckeria umbellata and *H. peltata* were first investigated by Johnson (1902), who showed that the development of the embryo-sac in these species followed the *Adoxa*-type. Schnarf (1931, 1936) and Maheshwari (1937) made a careful study of Johnson's figures and opined that the *Fritillaria*-form would be the actual course of development of the female gametophyte. This surmise is borne out by a reinvestigation of *Heckeria umbellata* by Maheshwari and Gangulee (1942). *H. sub-peltata* Kunth. is an uninvestigated species which grows in a wild condition in the evergreen forests of the Western Ghats. The results of a study of the development of the female gametophyte of this plant are presented in this paper.

OBSERVATIONS

The ovary and ovule present the same topographical and structural features as in *Heckeria umbellata* (Maheshwari and Gangulee, 1942). The archesporial cell is hypodermal in origin and cuts off a parietal cell, which divides only once in the majority of instances. The megaspore mother-cell enlarges and elongates lengthwise (Fig. 1). Its nucleus undergoes the characteristic pre-meiotic changes and divides into two nuclei (Figs. 2 and 3), which in turn complete the meiotic divisions by forming four megaspore nuclei which are not separated by walls ; the arrangement of the megaspore nuclei may be linear (Fig. 4) or more or less quadripolar (Fig. 5). At about this stage, one can notice a small vacuole appearing between the micropylar and the remaining three megaspore nuclei (Fig. 4). Finally the vacuole enlarges to such an extent so as to push away the micropylar nucleus and the three nuclei to opposite poles (Fig. 6). In this condition the nuclei begin to divide and the spindles of the three chalazally situated nuclei come still closer so that their equatorial regions lie more or less in a single line and plane (Fig. 7) ; but at the onset of anaphase, the

individuality of the three spindles is lost and they merge into a single large division figure, which produces two large triploid nuclei (Fig. 8). This stage of the embryo-sac, which shows two haploid nuclei at the micropylar and two triploid nuclei at the chalazal end, is the "Secondary four-nucleate stage". One more division of all the four nuclei (Fig. 9) results in an "8-nucleates" embryo-sac (Fig. 10), which,
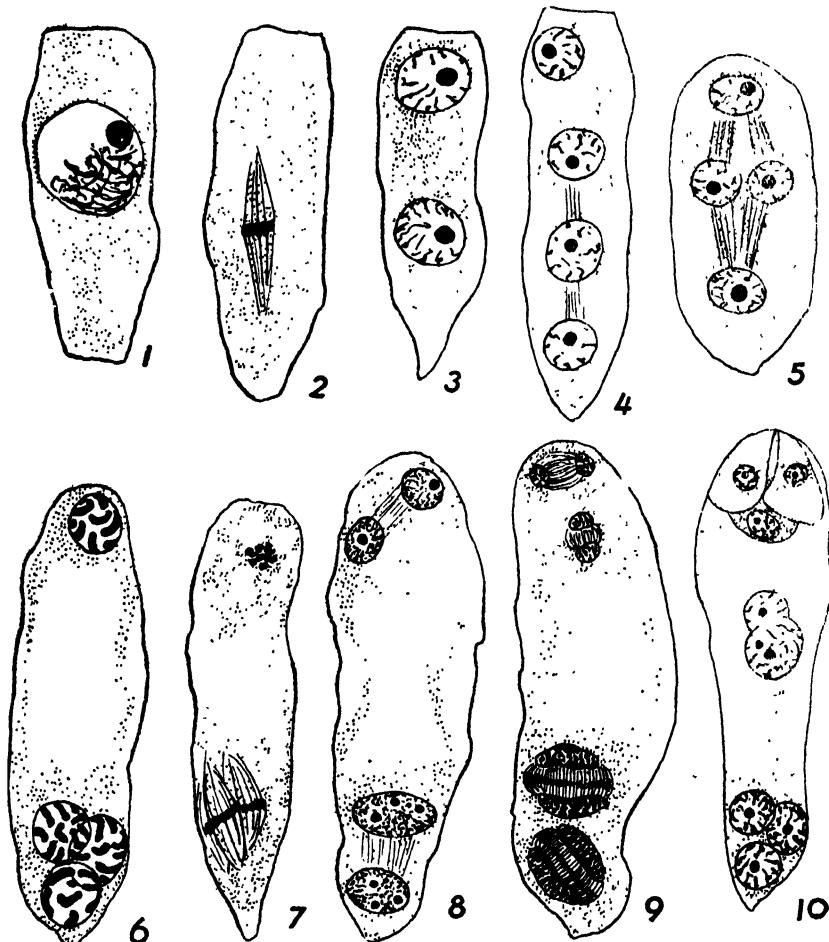
Figs. 1–10.—Fig. 1. Megaspore mother-cell. Fig. 2. Division of the mega-spore mother-cell. Fig. 3. 2-nucleate embryo-sac. Fig. 4. 4-nucleate embryo-sac in which the nuclei are arranged in a linear row. Note the vacuole between the micropylar nucleus and the rest. Fig. 5. 4-nucleate embryo-sac in which the nuclei are disposed in a quadripolar manner. Fig. 6. 1 plus 3 arrangement of the four nuclei. Fig. 7 Division of the "primary four nuclei" into the "secondary four-nucleate" embryo-sac; note the extremely juxtaposed spindles at the chalaza. Fig. 8. "Secondary four-nucleate embryo-sac." Fig. 9. The last (fourth) divi-sion of the embryo-sac nuclei. Fig. 10. Mature embryo-sac. All Figures × 900.

however, is equivalent to a tetrasporic 16-nucleate condition (*cf.* Swamy, 1944). The egg apparatus is organised by the haploid nuclei and the secondary embryo-sac nucleus is formed by the fusion of the haploid upper polar nucleus and the triploid lower polar nucleus.

SUMMARY

The female gametophyte of *Heckeria subpeltata* Kunth. has been studied and its development is shown to correspond to the *Fritillaria*-form.

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A NOTE ON THE LIFE-HISTORY AND THE SYSTEMATIC POSITION OF *RHINOSPORI-DIUM SEEBERI* (WERNICKE)

By H. CHAUDHURI, BALBIR SINGH AND KARTAR SINGH THIND

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VARIOUS cases of rhinosporidiosis on man, horse and cow have been reported from all over the world. In India it has been observed in Bengal, Madras, Poona and other parts (Allen, 1935 ; Allen and Dave, 1936 ; Anantnarayan Rao, 1938 ; Beattie, 1906 ; Cherian and Vasu Devan, 1929 ; Karunaratne, 1936 ; Krishna Murti, 1931 ; Kurup, 1931 ; Mandlik, 1937 ; Noronha, 1933 ; Norrie, 1929 ; Sahai, 1938).

In the present case the rhinosporidiosis has been studied on cow, bullock and pony. The material was obtained by one of the authors (Balbir Singh) from various places in C.P. The nasal polypi of these animals along with their fæces and nasal excretions were fully studied. The microtomic sections of the polypi were also prepared.

The systematic position of the causal organism, *Rhinosporidium seeberi* has been so far a disputed question. An attempt has been made in the present publication to throw some light on this.
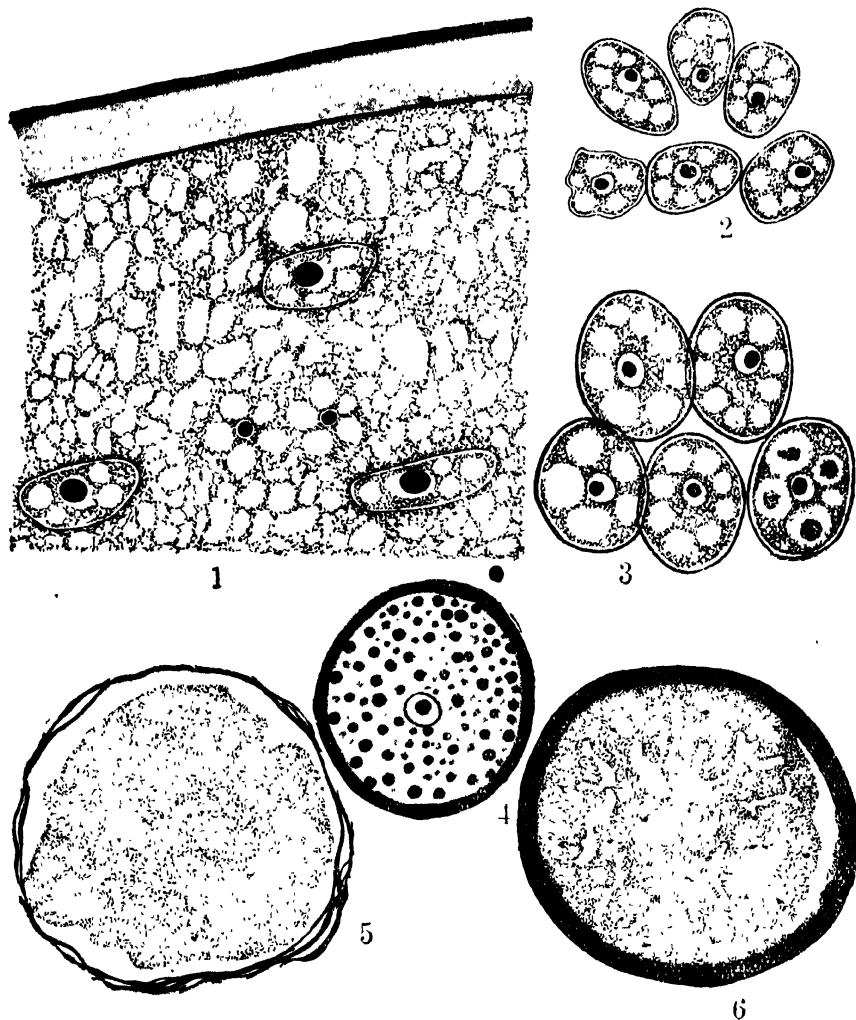
Young Stage

The parasite, as far as observed, starts its life-history with a small, spherical, oval or oblong body, sometimes with irregular boundary, inside the connective tissue cells of the polypus (Fig. 1). It measures 5-9 μ, the average being 6-8 μ in diameter. There is a nucleus with a distinct karyosome. The cytoplasm is granular and contains a few spherules (Figs. 2 and 3).

Trophic Stage

The parasite then enters a period of very active growth and considerably enlarges in size with corresponding accumulation of nutritive material in the form of spherical globules and increase in the size of the nucleus and the thickness of the wall (Figs. 4 and 12).

During the earlier part of the trophic stage the parasite remains more or less roundish and measures 13 μ to 65 μ in diameter. The wall is 1·3 μ to 5 μ in thickness and the size of the nucleus is 4 μ in a parasite which is 38 μ in diameter while it increases to 7 μ where the parasite attains the size of 60 μ in diameter. Later on the parasite becomes perfectly oval (Fig. 13) and measures from 91 μ to 130 $\mu \times 74 \mu$ to 78 μ. The thickness of the wall increases upto 9 μ as observed in a parasite

80 μ in diameter. In larger parasites the wall is comparatively thinner, being 7–8 μ thick.


Figs. 1–6.—Fig. 1. Section of the polypus with spores in the connective tissue cells. Fig. 2. Young spores showing envelope, the nucleus with karyosome, the cytoplasm and the vacuoles. Fig. 3. Spores at a later stage of development than in Fig. 2. Fig. 4. Trophic stage. Fig. 5. Showing reduction in the size of the spherules and globules. Fig. 6. The nuclei formed after mitotic division. Figs. 1–3, × 2700 ; Fig. 4, × 1500 ; Figs. 5 and 6, × 510.

An important change now takes place in the size of granules and spherules, which were much larger in the beginning but become reduced in size later on, just prior to the nuclear division (Fig. 5).

Nuclear division

After the maximum growth period the nucleus of the parasite shows' very active mitotic division.   Several thousand nuclei are thus formed before the commencement of the cytoplasmic division (Figs. 6–7).   The size of the parasite goes on enlarging and during the period of cytoplasmic division it varies from $144\,\mu$ to $109\,\mu \times 130\,\mu$ to $90\,\mu$.   The thickness of the wall is from 4-$9\,\mu$.
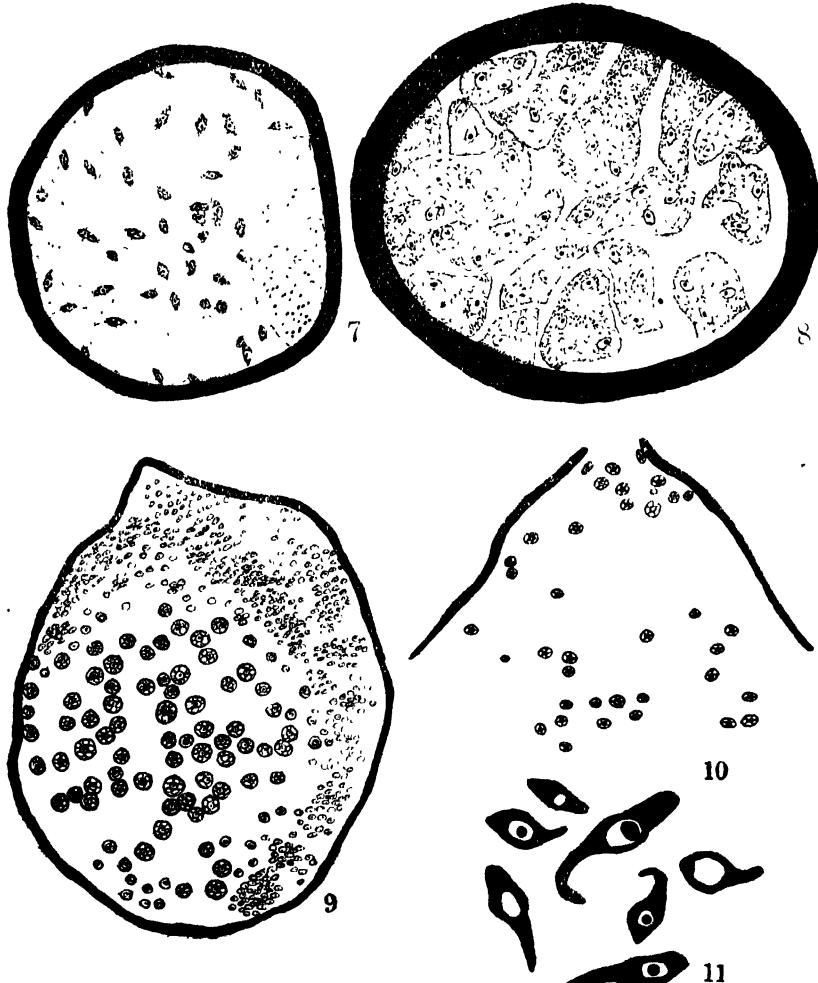
Figs. 7–11.—Fig. 7. More nuclei formed after mitotic division.   Fig. 8. Cytoplasmic division in progress. Fig. 9. Mature sporangium with beak at the top. Fig. 10. Pore differentiated [at the top of the sporangium. Fig. 11. Germination of spores giving rise to amœboid structures. Figs. 7 and 8, × 510 ; Figs. 9 and 10, × 700 ; Fig. 11, × 1620.

Formation of Spores

Cytoplasmic division now sets in which is fully illustrated in Fig. 8. It goes on till there are formed uninucleate protoplasmic masses (Fig. 9). These round off and a wall is laid down around each. The parasite now represents a young sporangium with numerous uninucleate spores (Fig. 9). These young sporangia vary in size from 187μ to $110\mu \times 156\mu$ to 110μ while the spores measure from 3–4μ in diameter. It is interesting to note that the central spores are differentiated earlier than the peripheral ones (Fig. 9).

The sporangia and the spores further increase in size: The fully ripe sporangia measure from 500μ to $400\mu \times 400\mu$, while the spores reach 6–9μ in diameter. These measurements are much higher than those given by Ashworth (1924).

Dispersal of Spores

At any point the wall of the sporangium may protrude out into a beak (Fig. 9). The beak later on breaks and a pore is formed through which the spores are discharged (Fig. 10).

Germination of Spores

Numerous spores from which blunt processes were seen in all stages of development were observed by the authors (Fig. 11). The amœboid structures thus formed seem to be the germinating spores of *Rhinosporidium seeberi* and were found in the nasal excretions. These no doubt bring about new infection.

It has, however, not been possible to carry out the artificial germination of these spores.

Systematic Position of Rhinosporidium seeberi

This organism was first seen by Seeber in 1896 in nasal polypi of man in Buenos Aires, which he described as a sporozoal parasite (Seeber, 1900) and Wernicke named this parasite as *Coccidium seeberi* in 1900. Belou (1903) in his treatise on animal parasitology described it as *Coccidium seeberi* Wernicke, 1900. Minchin and Fantham described *Rhinosporidium kinealyi* as a new genus and a new species from nasal polypi in man from India. Beattie (1906) also described *Rhinosporidium kinealyi* from Cochin material, obtained by Dr. Nair of Madras. Seeber's parasite is a *Rhinosporidium* and is the same as *R. kinealyi*. Fantham, Stephens and Theobold (1916) call it *R. kinealyi* (or *seeberi*). The question of priority of name has been discussed by Seeber (1912) and as pointed out by Hartmann (1921) the *Rhinosporidium seeberi* Wernicke has priority over *R. kinealyi*. From the nasal septum of a horse in South Africa, Zschokke (1913) described *R. equi*, a new species. That there is any specific difference between the human and equine form seems doubtful (Wenyon, 1926). All these authors regarded *Rhinosporidium* as a protozoa. Ridewood and Fantham (Fantham, 1907) in their classification put *Rhinosporidium* in subsection Polysporulea under Haplosporidia. Doflein (1906) also retained it in

Polysporulea but stated that it had many resemblances with *Chytridineæ*. Ashworth (1923) conclusively demonstrated that these were vegetable parasites. He gave a detailed account, calling it *Rhinosporidium seeberi* and related it with lower fungi for the following reasons :

(1) Presence of fatty reserves, (2) repeated nuclear division preparatory to spore formation, (3) division of cytoplasm at a later stage, (4) absence of residual cytoplasm, (5) presence of a mucoid substance between the spores, (6) wall being made up of cellulose and (7) formation of a definite pore in the sporangium.

As the thallus in *Rhinosporidium* is formed of a single cell and the mycelium is wholly lacking, Ashworth put the organism under Chytridineæ. The thallus of *Rhinosporidium seeberi* is holocarpic, *i.e.*, later on gives as a whole to the sporongium. So he put it in the family *Olpidiaceæ* of Chytridineæ.

The occurrence of germinating spores giving rise to amœboid structures as observed by the authors, indicates the affinity of *Rhinosporidium seeberi* to Chytridiales. The formation of zoospores has been suppressed here probably due to its peculiar mode of existence on man and other animals (Negroni, 1931).

Ainsworth and Bisby (1943) wonder if *Rhinosporidium* be put under *Endomycetales*. But from the evidence put forward it appears that it should be placed under *Chytridiales*.

It may be mentioned here that Anantnarayan Rao (1938), in his paper while giving a brief account of the organism, refers to both sporangia and asci. It seems that he has confused the two terms.

SUMMARY

Rhinosporidiosis occurring on cow, bullock and pony has been studied. The fæces and the nasal excretions were also examined. The amœboid structures formed from the germination of spores of *Rhinosporidium seeberi* were found in the nasal excretions. These structures further strengthen the affinity of *R. seeberi* with *Chytridiales*.

The authors have great pleasure in acknowledging their thanks to Dr. G. Watts Padwick, Imperial Mycologist, Imperial Agricultural Research Institute, New Delhi, for help with many references to literature and for kindly looking through the manuscript.

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Fig. 12. Section of Polypus (trophic stage). ×95

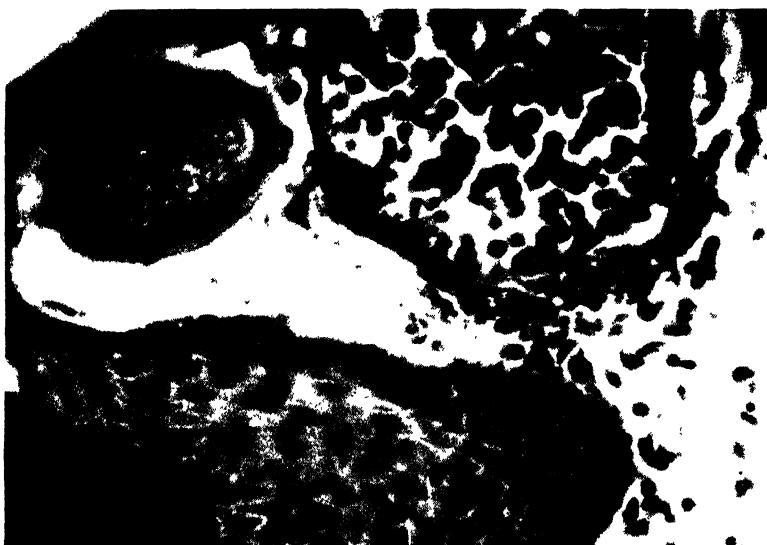Fig. 13. Section of Polypus with mature sporangium. 720

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STUDIES IN THE CÆSALPINIACEÆ

I. A Contribution to the Embryology of the Genus Cassia

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Our present knowledge of the embryology and cytology of the three families Mimosaceæ, Cæsalpiniaceæ and Papilionaceæ belonging to the order Leguminosæ (if we follow the classification of Hutchinson, 1926), chiefly on account of their different distribution, is very unequal. The Papilionaceæ being cosmopolitan and abundant both in Europe and N. America have received much attention, while the more tropical Mimosaceæ and Cæsalpiniaceæ have been only meagrely investigated. This fact has prompted the author to take up the study of the Cæsalpiniaceæ. The present paper deals with the structure and development of the ovule and embryo-sac of *Cassia* species. The author has started with this genus not only because it is the largest in the family and is represented by many species in this country, but also because the few observations that have been made by the earlier workers are in several cases contradictory.

Previous Work

The earliest reference to the embryology of the Cæsalpiniaceæ is found in the work of Braun (1860), who observed polyembryony in a species of *Cassia*. Later Guignard (1881) in his extensive studies on the embryology of the Leguminosæ also made some observations on the genera *Cæsalpinia, Cassia, Cercis, Gleditschia* and *Ceratonia*. He observed in *Cercis siliquastrum* both the chalazal megaspores often becoming 2-nucleate and each having the capacity of developing into a mature embryo-sac. Some further observations on the embryo-sac of *Cassia* were made by Hubert (1896).

Saxton (1907) worked out the structure and development of the ovule and embryo-sac of *Cassia tomentosa*. He observed a deeply situated primary archesporial cell which functions as the megaspore-mother cell without cutting off any primary wall cell. The megaspore-mother cell undergoes the two meiotic divisions in the normal manner and forms a linear tetrad of megaspores. The second megaspore from the chalazal end develops into the embryo-sac according to the *Normal* type. The mature embryo-sac at the chalazal end forms a tubular-extension which becomes filled with a row of antipodal cells, as happens in some Compositæ.

Ghose and Alagh (1933) studied *Cassia purpurea*. They found hypodermal primary archesporial cell in the ovules and the formation

of a primary wall cell. The second megaspore from the chalazal end, as in *Cassia tomentosa,* was found to develop into the embryo-sac.

Datta (1935) investigated *Cassia tora.* He found in the ovules sub-hypodermal primary archesporial cells, absence of the primary wall cells, and organisation of a linear tetrad of megaspores, out of which the chalazal one developed into the 8-nucleate embryo-sac. The antipodals, even though they were found to persist till fertilisation, remain only as free nuclei and are not organised into cells.

The latest work on the embryo-sac of the Cæsalpiniaceæ is a paper by Paul (1937) dealing with *Tamarindus indica.* He reports the differentiation of the primary archesporium from the sub-hypodermal layer, formation of the primary wall cell and a normal tetrad of megaspores from the megaspore-mother cell. The chalazal megaspore is the functional one and develops into the embryo-sac according to the *Normal*-type.

MATERIAL AND METHODS

During the course of the present investigation material of the following species of Cassia has been investigated.

1. *C. occidentalis* Linn.
2. *C. abtusifolia* Linn.
3. *C. glauca* Lamk.
4. *C. glauca* Lamk. var. *suffruticosa* Koenig.
5. *C. marginata* Roxb.
6. *C. siamea* Lamk.

The first two species grow abundantly at Benares, particularly during the rainy season in waste places, and their material was collected from plants growing wild in the Benares Hindu University area. The material of *Cassia glauca* was obtained from a plant cultivated in the Benares Hindu University Botanical Garden, and that of *C. glauca* var. *suffruticosa* from a plant growing in the Sri Sita Ram Krishishala, Benares. The material of *C. marginata* was collected by Dr. A. C. Joshi from a tree growing in one of the gardens at Allahabad and that of *C. siamea* from trees planted on road-sides in the Benares Hindu University campus.

In all cases the material was fixed in Nawaschin's fixative between 12 noon and 3 p.m. during the months of September, October and November, 1940. An exhaust syringe was employed to cause the rapid immersion of the material in the fixative ; 12–18 hours after fixing, the material was rinsed in water four or five times and then transferred to 70 % alcohol. The further dehydration and embedding in paraffin was carried out according to the customary methods. Sections were cut 8–16μ thick. Delafield's Hæmatoxylin and Newton's Iodine Gentian Violet were employed as stains.

STRUCTURE AND DEVELOPMENT OF THE OVULE

The ovules in all species of *Cassia* are borne in two rows along the ventral suture of the monocarpellary unilocular gynœcium, the ovules

of the two rows alternating with one another.   The number of ovules
in a carpel varies slightly with each species, but it may be stated that
on the average there are 30–50 ovules in a carpel.
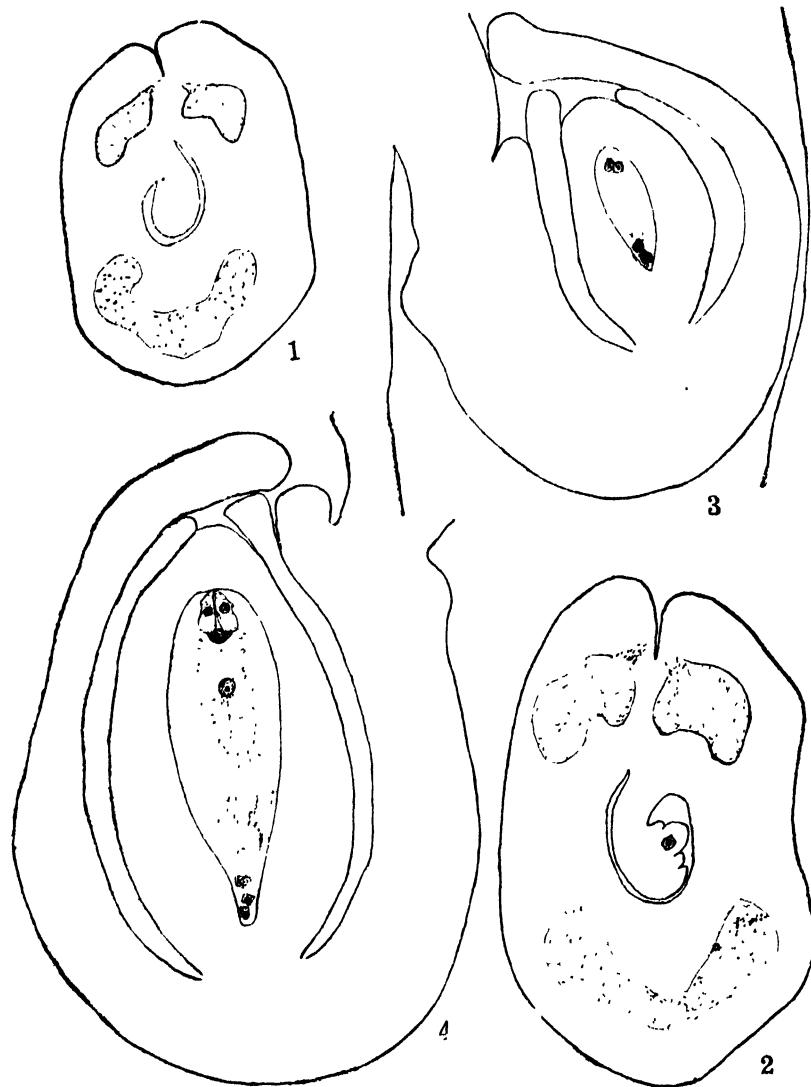
Figs. 1–4.   *Cassia occidentalis.*—Figs. 1 and 2.   Transverse sections of young
ovaries showing early stages in the development of the ovule.   The vascular bundles
of the carpel are stippled.   Fig. 3.   An ovule at the 4-nucleate embryo-sac stage.
Fig. 4.   An ovule at the mature embryo-sac stage.   Figs. 1 and 2, × 800 ;   Figs. 3
and 4, × 150.

The ovules first arise as small papillæ from the margins of the carpel, which has at this stage the form of a linear structure folded upwards along the midrib, a fact which agrees with the classical interpretation of carpel morphology. The two margins of the carpel are still free from each other and the carpel is open on the posterior side (Fig. 1). The development of the ovule primordia results chiefly from the activity and rapid division of groups of hypodermal cells, and after their differentiation these primordia are seen protruding into the ovary cavity (the space enclosed by the wall of the carpel). The ovule primordia at first are quite straight, but soon during further growth the cells develop more actively on one side than on the other. Consequently the young ovules bend towards the apex of the ovary, and gradually assume an anatropous form (Fig. 3). Reeves (1930) in *Medicago* observed that the curvature of the ovules is conditioned by mechanical pressure. He found as long as there is space for free development, the ovule remains orthotropous, but as soon as the ovule during its growth comes in contact with the dorsal wall of the carpel opposing it, its straight growth comes to end and it curves generally towards the base. Maheshwari (1931) in *Albizzia Lebbek* describes the young nucellus as growing at first straight and at right angles to the placenta (ventral suture), but when it approaches the dorsal wall of the carpel it begins to curve upwards. Singh and Shivapuri (1935) describe the same condition in *Neptunia oleracea*, a member of the Mimosaceæ. In a few cases, in which the carpel was found to remain open throughout its development, the ovules were found to remain permanently orthotropous. Great significance has been attached to this fact by Joshi (1935) in the evolution of the anatropous form of the ovule. In *Cassia* species studied during the course of the present investigation, however, no such relation has been found. The primordia of the ovules begin to bend towards the apex of the ovary even when these are quite away from the dorsal wall of the ovary.

The mature ovules in all *Cassia* species, even after the development of embryo, are anatropous with a slight tendency towards amphitropy (Figs. 3 and 4). They possess two integuments. The inner integument in the flowering plants generally differentiates from the ovule primordium almost simultaneously with the differentiation of the primary archesporium, but in all *Cassia* species investigated by the writer it did not appear till the primary archesporial cell had cut off the primary wall cell and had reached the megaspore-mother cell stage. The development of the integuments thus in the genus is considerably delayed. The primordium of the inner integument arises just below the level of the megaspore-mother cell (Fig. 2). Soon after its differentiation, the primordium of the outer integument appears just below that of the inner integument. In spite of the late start, the outer integument soon outgrows the inner by its faster development, so that by the time of tetrad formation the outer integument has attained a slightly greater length than the inner (Figs. 25 and 31). While the outer integument by this time has reached the level of the nucellus apex, the inner integument is seen to end somewhat below the level of the nucellus. The disparity between the growth of the two integuments is maintained
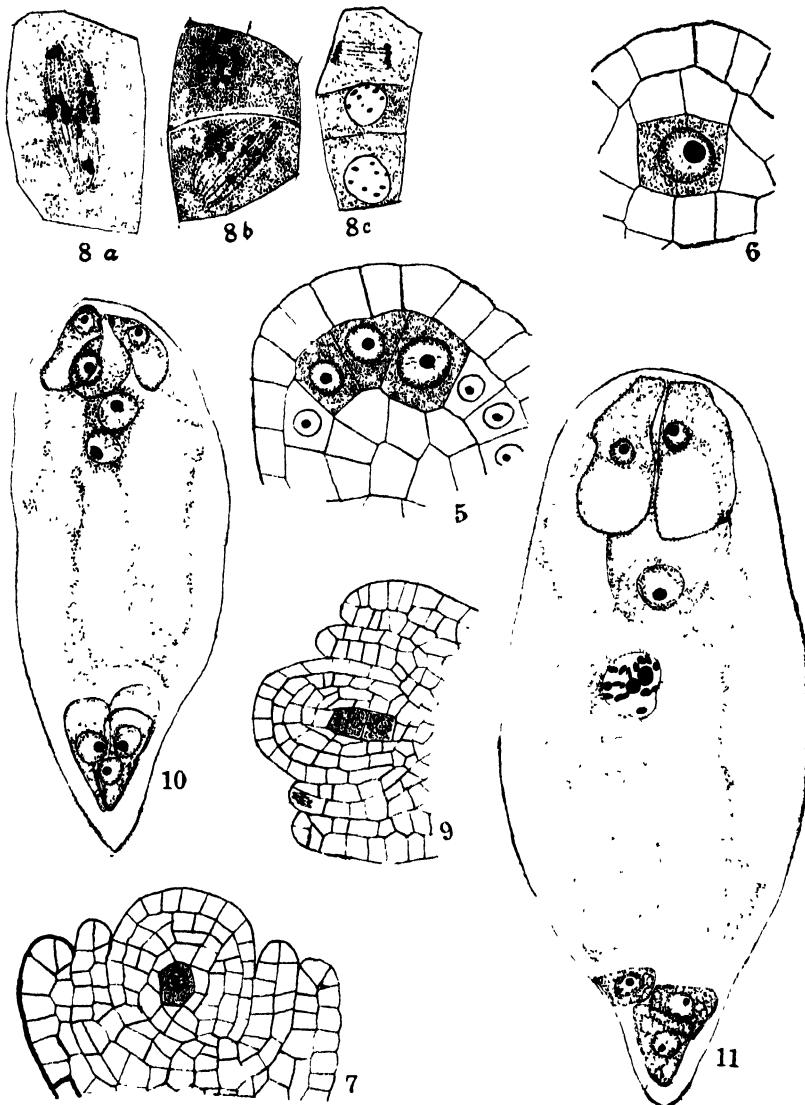
Figs. 5–11. *Cassia occidentalis.*—Various stages in the development of the embryo-sac.—Fig. 5. Apex of the nucellus showing a group of primary archesporial cells. Fig. 6. Formation of the primary wall cell and its division by an anticlinal wall. Fig. 7. An ovule showing the megaspore-mother cell. Fig. 8 *a–c.* Three stages in the development of a tetrad of megaspores ; (*a*) I meiotic division in the megaspore-mother cell ; (*b*) the II meiotic division ; (*c*) a stage showing the formation of a T-shaped tetrad of megaspores. Fig. 9. The ovule from which the T-shaped tetrad shown in Fig. 8 *c* has been sketched. Fig. 10. A young 8-nucleate 7-celled embryo-sac. Fig. 11. A mature embryo-sac after the fusion of the polar nuclei. Figs. 7 and 9, × 800 ; the rest, × 1700.

even in the later stages, so that in the mature ovule (an ovule at the time of fertilisation) the micropyle is mostly formed by the outer integument. The inner integument contributes only to a very small length of the micropyle (Fig. 4). There are two further peculiarities of the micropyle. Firstly, the passage formed by the outer integument is not quite opposite to that formed by the inner. It is rather to one side, so that the micropyle is not straight but somewhat zig-zag. Secondly, at the micropyle the outer integument is never in direct contact with the inner integument. In this region there is always a small space between the two integuments. Both the integuments in all species are two layers of cells thick except near the micropyle, where both the integuments are 4–5 cells thick.

The nucellus in species of *Cassia* is massive from the very beginning. At the tetrad stage there are approximately 4–5 layers of nucellus cells above the tetrad, 3–5 layers on the sides, and 4–5 layers beneath the tetrad (Figs. 9 and 31). By the time the embryo-sac reaches the 4-nucleate stage the number of cells in the nucellus above the embryo-sac has increased to 8–10 layers due to divisions in the parietal cells. Before fertilisation many of these parietal cells are gradually crushed by the growing embryo-sac, but the number of cell layers above the micropylar end of the embryo-sac remains the same due to periclinal divisions in the epidermal cells of the nucellus. As the parietal cells are crushed at this end, the epidermal cells divide to restore the number of layers destroyed. This growth gives rise to considerable pressure inside the ovule, so that the epidermal cap shortly before fertilisation begins to project as a small beak into the micropyle of the ovule (Fig. 4). This pushes outwards the inner integument and leads to considerable decrease in the size of the air-space found between the two integuments close to the micropyle of the ovule. The formation just before fertilisation of an epidermal cap at the micropylar end of the nucellus with a small beak projecting into the micropyle seems to be a characteristic feature of all the Cassias examined by the author. I have seen it also in a number of other Cæsalpiniaceæ and perhaps this feature is characteristic of the whole family. There are approximately 6–7 layers of cells below the chalazal end of the embryo-sac, and 7–8 on the sides of the embryo-sac at the time of fertilisation. To a large extent these cell layers are soon crushed by the post-fertilisation growth of the embryo-sac.

Another characteristic feature of the ovule of Cassias is that the epidermis of the funicle and the adjacent part of the outer integument on the outer side (*i.e.*, the side on which the ovule does not bend) remains meristematic for a long time. At the tetrad stage these cells are quite distinct from the other cells of the ovule, possessing as they do dense cytoplasm and no conspicuous vacuoles. Further close to the hilum these cells grow out into a short hump-like outgrowth, which persists throughout the life of the ovule.

DEVELOPMENT OF THE EMBRYO-SAC

As the ovule begins to curve, but before the appearance of the integument primordia, the primary archesporium differentiates from

the other cells of the nucellus. In the flowering plants in general the curving of the ovule, the appearance of the integument initials and development of the primary archesporium are almost synchronous.
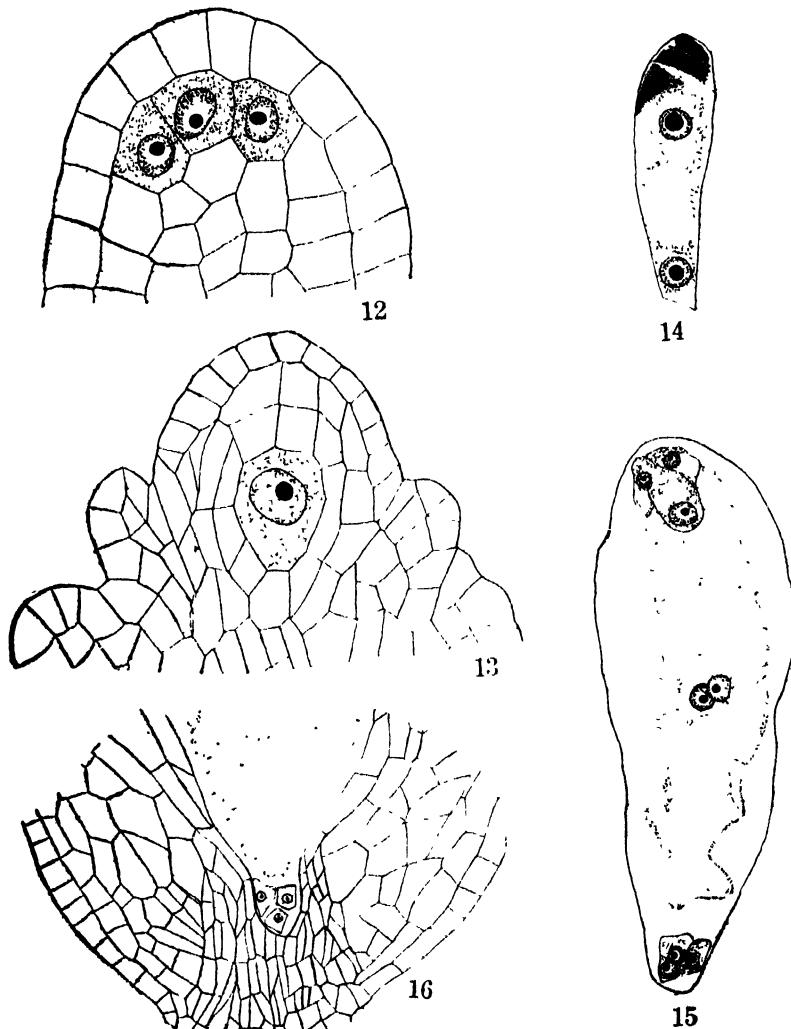
Figs. 12–16. [*Cassia obtusifolia.*—Fig. 12. Nucellus showing a group of archesporial cells. Fig. 13. An ovule at the megaspore-mother cell stage. Fig. 14. A 2-nucleate embryo-sac, with three degenerating micropylar megaspores. Fig. 15. An 8-nucleate, 7-celled embryo-sac. Fig. 16. Antipodal region of an embryo-sac showing the tubular extension of its chalazal end. Fig. 15, × 1400 ; the rest, × 1700.

In all Cassias, however, as has been stated before, the integuments begin to develop rather late, only after the differentiation of the mega-spore-mother cell in the ovules. The primary archesporium in all species

was found to be of hypodermal origin. In *Cassia glauca* and *C. glauca* var. *suffruticosa* a single primary archesporial cell is quite prominent from an early stage and can be easily distinguished from the surrounding cells (Figs. 17 and 22). In other species all the cells of the hypodermal layer are equally prominent and look just alike. They all show dense cytoplasm and possess almost equally large nuclei (Figs. 5, 12 and 26). One of these cells, however, generally the most centrally situated one, divides by a periclinal wall into an outer parietal cell and an inner megaspore-mother cell. This cell may be said to act as the primary archesporial cell (Figs. 6, 13, 18, 23, 29 and 30).

Describing the primary archesporium of the ovule, Coulter and Chamberlain (1903) state : "The archesporium is recognized by the increasing size and different reaction to stains of one or more hypodermal cells. Doubtless all of the hypodermal cells are potentially archesporial, and there is reason for believing that the deeper cells of the nucellus, most of which are probably derivatives from the original hypodermal layer, may be also. In the vast majority of the cases, however, only cells of the hypodermal layer show those changes that are characteristic of archesporial cells. It is not always easy to determine just how many hypodermal cells are to be included in the archesporium, for there is often complete gradation from cells with the size and staining reaction of undoubted archesporial cells to those showing neither increase in size nor the characteristic staining reaction. This is to be expected in case all the hypodermal cells are potentially archesporial, and there is no definite point in its history when such a cell ceases to be merely hypodermal and becomes clearly archesporial." While examining the ovules of the different species of *Cassia* for the primary archesporial stages, I have felt exactly like Coulter and Chamberlain. In the beginning in most species all the hypodermal cells at the apex of the nucellus are just similar. Then one of them cuts off a parietal cell and may be said to function as stated above as the primary archesporial cell.

Saxton (1907) noted in *Cassia tomentosa* that the primary archesporial cell is deep-seated, *i.e.*, sub-hypodermal and functions directly as the megaspore-mother cell without cutting off any parietal cell. Datta (1935) has described the same feature in *Cassia tora*. From the uniform hypodermal origin of the primary archesporium that I have noticed in the species examined by me, I am led to believe that the observations of both these authors are probably incorrect. This error has been made by them very likely from an examination of too old material, in which the parietal tissue had already begun to develop. I have not been able to see the paper by Saxton, but from examining the figures of Datta I find the Fig. 3 of his, which is almost at the same stage as Fig. 2 (and the latter is supposed to represent the primary archesporium). In the material examined by me the ovule has always developed up to the megaspore-mother cell stage by the time the integument primordia differentiate. Further, *Cassia obtusifolia* examined by me is very closely related to *C. tora*. In *Cassia obtusifolia*, I have clearly seen the hypodermal origin of the primary archesporium and the formation of a primary parietal

cell. It is not possible to believe that two such closely related species can show such a great difference in their embryological characters. What Datta regards as the primary archesporial cell is really the megaspore-mother cell after the cutting off of the primary wall cell. The observations of Ghose and Alagh (1930) on *Cassia purpurea* agree with mine. They also noted hypodermal archesporium and the formation of the primary wall cell. This character, therefore, may be taken as characteristic of the genus. Paul (1937) has reported sub-hypodermal origin of the primary archesporium in *Tamarindus indica*. I consider his observations also doubtful.
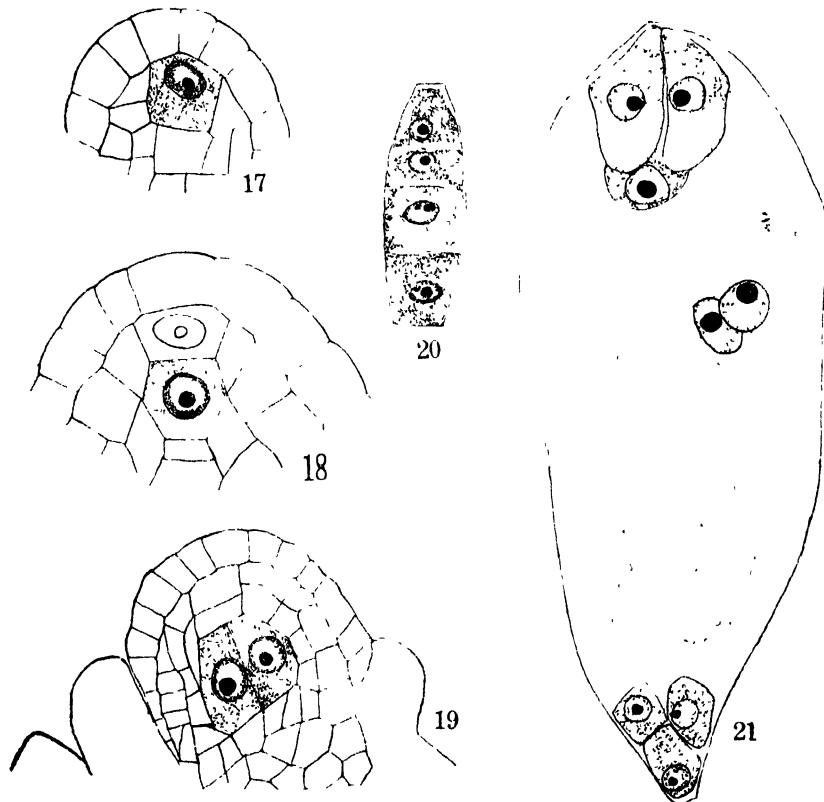
Figs. 17–21. *Cassia glauca.*—Fig. 17. The primary archesporial cell. Fig. 18. The differentiation of the primary wall cell. Fig. 19 An ovule showing two megaspore-mother cells. Fig. 20. A linear tetrad of megaspores. Fig. 21. A mature embryo-sac. Fig. 19, × 800 ; the rest, × 1700.

One functional archesporial cell and one megaspore-mother cell is the general character of the ovules of the different Cassias, but the occasional occurrence of two megaspore-mother cells has been observed in *Cassia glauca* (Fig. 19), *C. glauca* var. *suffruticosa* and *C. siamea*. Perhaps such exceptional cases are likely to occur in other species also

if a larger amount of material is examined. However, whenever two megaspore-mother cells were observed in an ovule, only one was seen to develop up to the tetrad stage. I did not come across any case of two tetrads or multiple embryo-sacs in an ovule. Occurrence of more than one megaspore-mother cells in an ovule has been previously noted by Datta (1935) in *Cassia tora*, and there are many similar instances reported among other Leguminosæ, *e g.*, *Albizzia Lebbek* of the Mimosaceæ (Maheshwari, 1931), *Medicago sativa* (Reeves, 1930), *Melilotus alba* (Cooper, 1933), etc., belonging to the Papilionaceæ.

The primary parietal cell divides in all planes and by the time the two meiotic divisions in the megaspore-mother cell are completed, it gives rise to 4–5 layers of parietal cells (Figs. 9 and 31). Later the number of these layers increases to 8-10. Such extensive development of the parietal tissue seems to be characteristic of the Cæsalpiniaceæ and Mimosaceæ In the Papilionaceæ, on the other hand, the parietal tissue is poorly developed. This agrees with the primitive character of the first two families and the more advanced position of the last family in the order.

The megaspore-mother cell after its differentiation undergoes a considerable period of rest and growth without any nuclear changes. It increases considerably both in length and breadth. The ovule also increases considerably during the megaspore-mother cell stage, so that the megaspore mother-cell becomes deep-seated. The meiotic divisions in all investigated species proceed in the normal manner. In *Cassia occidentalis* $14n$ chromosomes were counted during these divisions (Fig. 8 *a*). After the first meiotic division the mother cell is divided into two dyads by a transverse wall, which does not lie exactly in the middle (Figs. 8 *a*, *b* and *c*). The dyads are thus of unequal size, the chalazal one being larger. The second meiotic division in the two dyad cells generally does not proceed simultaneously. It starts earlier and proceeds more actively in the chalazal dyad than in the micropylar, so that in some cases even when the division has been completed in the chalazal dyad, the micropylar dyad is in the telophase stage (Fig. 8 *c*). Due to the difference in the size of the dyads, the megaspores formed from them also show slight size differences. The two chalazal megaspores are slightly larger than the two micropylar ones. The four megaspores are generally arranged in a linear order (Figs. 20, 24 and 31), but a T-shaped arrangement of the megaspores (Figs. 8 *c* and 9) was also seen in several instances in almost all species. In addition to this variation, in one ovule of *Cassia glauca* var. *suffruticosa* one megaspore-mother cell was observed to have given rise to an iso-bilateral tetrad of megaspores (Fig. 25). In this ovule there were two megaspores. One of these had formed this exceptional type of tetrad. The other was still in the megaspore-mother cell stage. It is not illus-trated in the figure. Exceptional occurrence of isobilateral tetrads of megaspores in the flowering plants has been previously observed by Ducamp (1902) in *Fatsia japonica*, Greco (1930) in *Myrtus communis*, and Capoor (1937) in *Urginea indica*.

In all the species studied during the course of the present investigation the chalazal megaspore is found to develop into the embryo-sac (Figs. 14 and 31). The other megaspores degenerate, but the traces of the degenerating cells may be seen up to the 2-nucleate stage of the
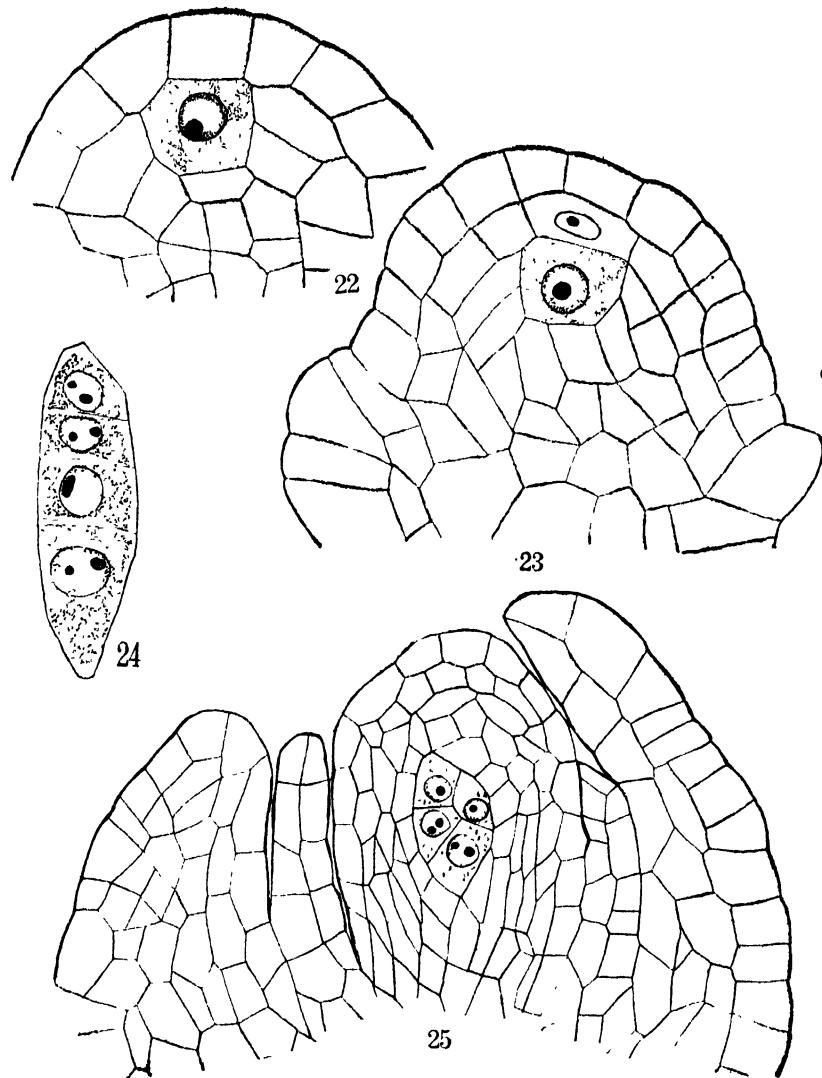
Figs. 22–25. *Cassia glauca* var. *suffruticosa.*—Fig. 22. The primary archesporial cell. Fig. 23. Formation of the primary wall cell. Fig. 24. A linear tetrad of megaspores. Fig. 25. An ovule showing an isobilateral tetrad of megaspores. Fig. 25, × 900 ; the rest, × 1700.

embryo-sac. Datta (1935) observed the same feature in *Cassia tora*. but Saxton (1907) and Ghose and Alagh (1933) found in *C. tomentosa* and *C. purpurea* respectively the second megaspore from the chalazal end developing into the embryo-sac. Such variation in the selection of the megaspores is common in the whole order Leguminosæ and was observed as early as 1881 by Guignard. From a study of about 40 species he concluded that in the Leguminosæ of the four megaspores of the tetrad either the innermost or the one next to it is the functional one.

The functional megaspore develops into the embryo-sac according to the *Normal*-type. It increases in size. Along with this vacuoles develop both above and below the central nucleus. The latter divides. The daughter nuclei move to the two poles of the embryo-sac and a central vacuole becomes prominent. The two nuclei at the poles undergo two more mitotic divisions, so that an 8-nucleate embryo-sac is formed with four nuclei at either end. Three nuclei at the micropylar end organise into the egg-apparatus, three at the chalazal end into antipodals and the two polar nuclei are left in the central cell (Figs. 10, 15, 21, 28 and 32).

Both the egg and the two synergidæ are nearly pyriform. The egg is slightly larger than the synergidæ. It shows a large vacuole towards the micropylar end, while the nucleus and the cytoplasm are pressed towards the chalazal end. The synergidæ show a large vacuole in the chalazal half, while the micropylar half is densely filled with cytoplasm. The nucleus is found embedded in the cytoplasm just above the vacuole. In all species the synergidæ show prominent hooks and a distinct "filiform apparatus" at the time of fertilisation (Figs. 11, 21, 28 and 32).

The antipodals form definite cells (Figs. 10, 11, 21, 28 and 32). Datta (1935) reports that in *Cassia tora* the antipodals are not organised into cells but remain as free nuclei. As I have observed antipodal cells in all the species investigated by me, his observations appear to me quite erroneous. Even in one of his own figures he has represented one of the antipodals as a cell with a cell-wall around it. In all the Leguminosæ investigated so far the organisation of antipodal cells has been noted. The antipodals in all Cassias are quite prominent and persist till the time of fertilisation. They often develop large vacuoles. In *Cassia glauca* var. *suffruticosa* they are sometimes even more prominent than the egg-apparatus. In *Cassia tomentosa*, Saxton (1907) mentions the presence of more than three antipodals, but I have not come across any such case in my material.

The two polar nuclei meet near the egg-apparatus or the middle of the embryo-sac. Here they remain together for a long time, but fuse only just before fertilisation. In *Cassia occidentalis*, the two polar nuclei just before fusion have been observed to enter the prophase stage and show the chromosomes quite distinctly (Fig. 11).
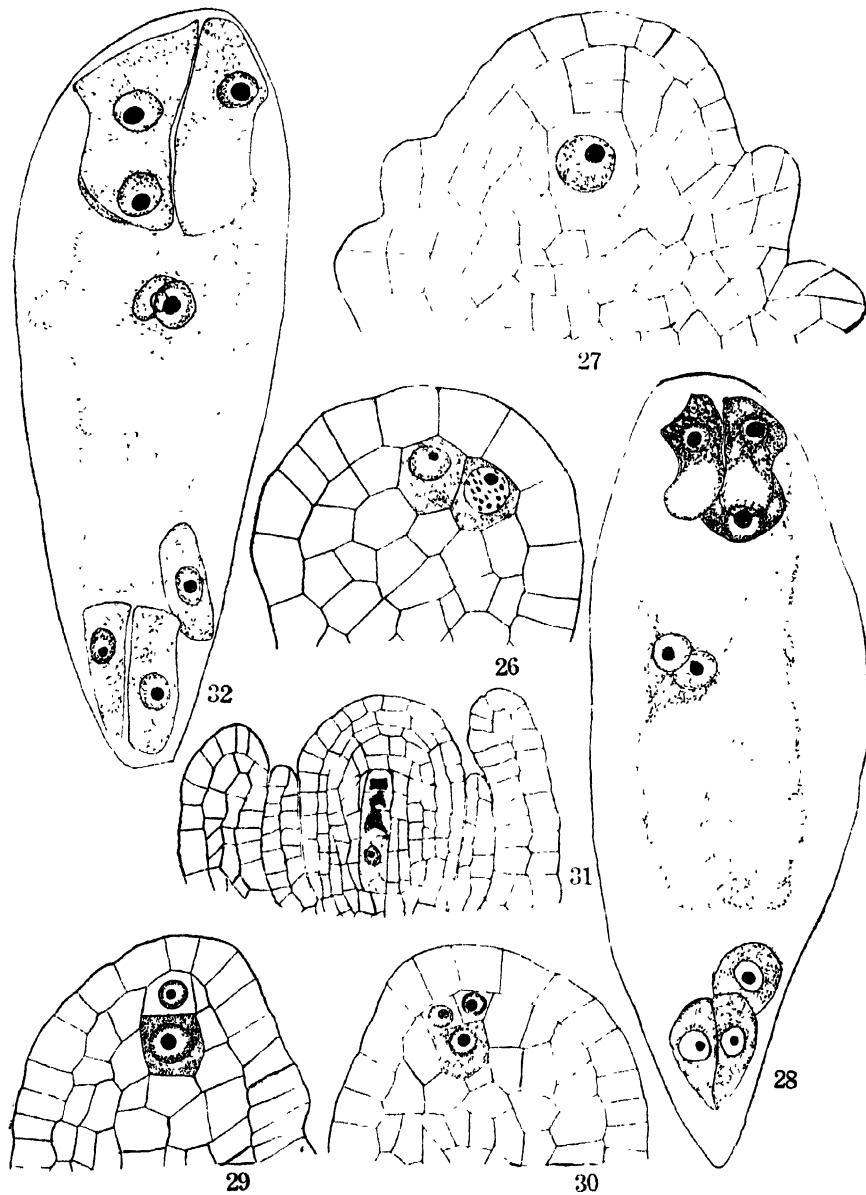
Figs. 26–32.—Figs. 26–28. *Cassia marginata.*—Fig. 26. A group of primary
archesporial cells. Fig. 27. Apical region of an ovule with a megaspore-mother
cell. Fig. 28. Mature embryo-sac. × 1700. Figs. 29–32. *Cassia siamea.*—Fig. 29.
An ovule showing the differentiation of a primary wall cell and the megaspore-
mother cell. Fig. 30. The same as Fig. 29 but showing the anticlinal division of
the primary wall cell. Fig. 31. An ovule showing a linear tetrad of megaspores.
Fig. 32. A mature embryo-sac. Fig. 31, × 900 ; the rest, × 1700.

SUMMARY

The development of the ovules and embryo-sac has been studied in *Cassia occidentalis* Linn., *C. obtusifolia* Linn., *C. glauca* Lamk., *C. glauca* Lamk. var. *suffruticosa* Koenig., *C. marginata* Roxb. and *C. siamea* Lamk. The ovules in all species are anatropous, with a slight tendency towards amphitropy, and bitegmic. The integument initials appear only after the primary archesporial cell has cut off the primary wall cell. The micropyle is somewhat zigzag and is formed largely by the outer integument. Further, in the region of the micropyle the outer integument for a short distance is separated from the inner by a small air-space. The nucellus is quite massive. The formation just before fertilisation of an epidermal cap at the micropylar end of the nucellus with a small beak projecting into the micropyle is characteristic. The epidermis of the funicle and the adjacent part of the outer integument on the outer side remains meristematic for a long time and close to the hilum grows out into a short hump-like structure, which persists throughout the life of the ovule.

The primary archesporium in all species is hypodermal and a primary wall cell is always formed. The earlier records about the occurrence of sub-hypodermal archesporium in some species of *Cassia* appear to be all doubtful. The megaspore-mother cell gives rise to a linear or T-shaped tetrad of megaspores, of which the chalazal develops into an 8-nucleate embryo-sac according to the normal type. In one instance in *C. glauca* var. *suffruticosa* an isobilateral tetrad of megaspores has been observed. The synergidæ are prominently hooked and show the filiform apparatus. The egg is pyriform and slightly larger than the synergidæ. The antipodals are definite cells and persist till the time of fertilisation. The two polar nuclei meet near the egg-apparatus. They fuse only just before fertilisation.

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THE PLACE OF ANGIOSPERM EMBRYOLOGY IN RESEARCH AND TEACHING*

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IN the history of Angiosperm Embryology there have been three distinct periods : the *first* in which the chief aim was to unravel the fundamental facts regarding the development of the pollen and embryo-sac, and the processes of fertilisation and seed formation ; the *second* in which interest centred largely round a study of comparative embryology and an evaluation of the data thus obtained for the improvement of the existing systems of classification ; and the *third* and most recent in which Embryology has become an experimental science like Physiology and Cytology, where one tries to study such problems as the storage of pollen and its germination, the receptivity of the stigma, fertilisation and fruit-setting, etc., and the optimum conditions required for them.

DESCRIPTIVE EMBRYOLOGY

It is not necessary to spend much time on the first of these, *i.e.*, Descriptive Embryology, as most of the facts relating to the course of development of pollen, embryo-sac, endosperm and embryo had become clear towards the close of the last century through the efforts of Amici, Schleiden, Hofmeister, Strasburger, Treub, Guignard, Nawaschin and others, and are now a commonplace in all textbooks of botany. A very good summary of this work was given by Coulter and Chamberlain in the year 1903 and it was followed later by the publication of Schnarf's (1929) "Embryologie der Angiospermen", which is at present the most important and exhaustive treatise on this subject. Although little that is fundamentally new has probably been discovered since then, many errors and misinterpretations made by previous workers have been corrected and a mass of valuable information has been added regarding certain details concerned with the formation of the male gametes, the types of embryo-sac development, the cytology of fertilisation, the origin and function of endosperm haustoria and the development of the embryo. Work of this type is still in progress but the results will not be proportionate to the time spent unless a worker devotes his attention to just one aspect of the life-history in which he is most proficient and studies this in as many plants as possible. It is in this way that Finn in the Ukraine and Wulff in Germany and recently also some workers in the U.S.A. have been able to discover a number of important facts on the structure and development of the male gametes and Souèges in France on the development of the embryo in a large number of angiospermous families.

Phylogenetic Embryology or Embryology in relation to Systematic Botany

In the second period, which may be said to have commenced with the beginning of this century, embryology began to be used as an aid in the improvement of our systems of classification, the most important contributions in this line having come from Sweden (Stockholm, Uppsala and Lund), Germany (Bonn, Berlin and Vienna), and the U.S.A. (Chicago, Baltimore and California). A great impetus was given to such studies by the publication of Schnarf's excellent handbook entitled "Vergleichende Embryologie der Angiospermen" in which the author has summarised the existing state of our knowledge of the embryology of each family and added a number of valuable suggestions and comments at the end of each order. Most of the embryological work done in India has followed the publication of this book, to whose author we owe a debt of gratitude which cannot be expressed adequately in words. Although I alone among the Indian embryologists have had the privilege of working with Prof. Schnarf, yet all have gained considerable inspiration and insight into this difficult field through the medium of his publications.

As the value of embryology in questions relating to systematic botany does not appear to be sufficiently appreciated in this country by those who are engaged in other lines of study, it is necessary to consider this aspect in some detail.

It is a matter of common knowledge that on the basis of external morphology of the vegetative organs a genetical relationship may sometimes be inferred to exist between plants which belong to widely separated groups (*Equisetum* and *Casuarina* ; *Ephedra* and certain Asclepiads ; some cacti and Euphorbias). Taxonomists therefore take recourse to the flower as it is a more conservative organ than the stem and leaf. But, if we assume phyletic trends in the external morphology of the flower, why not in the internal structures, for these must be still more conservative (being less amenable to environmental influences) and therefore of special value in judging the proper position of certain doubtful groups ? I am told that the zoologist would ordinarily refuse to assign an unknown animal to its systematic position until he has had an opportunity of examining its internal organs. No one can doubt that the same should be done with plants also, and if anything has prevented *us* from applying the anatomical and embryological method on a large scale, it is only the greater labour involved in it. The work *has* however to be undertaken now on a larger scale than ever as the systematist has taken us almost as far as he could towards our goal of a natural system of classification and can hardly make much headway without our help and co-operation.

Before proceeding further I must now enumerate such characters in the embryology of an angiosperm which are usually considered to be of major value in delimiting the larger plant groups :—

1. *Anther tapetum.*—Whether it is of the glandular or the amœboid type.

2. *Quadripartition of the microspore mother-cell.*—Whether it takes place by furrowing or by the formation of cell-plates.

3. *Development and organisation of the male gametophyte.*—Number and position of the germ pores and furrows ; adornments of the exine ; place of formation of the generative cell ; number and shape of the nuclei in the pollen grain at the time of its discharge from the anther.

4. *Development and structure of the ovule.*—Number of integuments and the alterations in structure which they undergo during the formation of the seed ; presence or absence of vascular bundles in the integuments ; shape of the micropyle, whether it is formed by the inner integument or the outer or both ; the presence or absence of an obturator.

5. *Form and extent of the nucellus.*—Whether it is broad and massive or thin and ephemeral; presence or absence of a hypostase; the place of origin of the integument or integuments, whether close to the apex of the nucellus (as in the Rubiaceæ) or near its base (as in the Orchidaceæ) ; persistence or disappearance of the nucellus in the seed.

6. *Origin and extent of the sporogenous tissue in the ovule.*—Nature of the archesporium, whether it is one-celled or many-celled ; presence or absence of wall layers ; the presence or absence of periclinal divisions in the cells of the nucellar epidermis.

7. *Megasporogenesis and development of the embryo-sac.*—*i.e.*, to which of the following main types or its modifications does it correspond : Normal, *Oenothera, Allium, Peperomia, Fritillaria, Adoxa, Plumbago, Plumbagella,* etc. ?

8. *Form and organisation of the mature embryo-sac.*—Shape of the embryo-sac and the number and distribution of its nuclei ; an early disappearance or otherwise of the synergids and antipodal cells ; increase in number of antipodal cells, if any ; formation of haustoria, if any, from some part of the embryo-sac.

9. *Fertilisation.*—The path of entry of the pollen tube ; the interval between pollination and fertilisation ; any tendency towards a branching of the pollen tubes during their course to the ovule.

10. *Endosperm.*—Whether it is of the nuclear, cellular or Helobiales type, and direction of laying down of the first wall in such cases where it is cellular ; presence or absence of endosperm haustoria and the manner in which they are formed if present ; nature of food reserves in endosperm cells.

11. *Embryo.*—Relation of the proembryonal cells to the body regions of the embryo ; form and organisation of the mature embryo ; presence or absence of suspensor haustoria.

12. *Certain abnormalities of development.*—Apomixis, polyembryony, parthenogenesis, etc.

While these are the most important characters usually taken into account in systematic studies, there are many others which it is

difficult to put down in writing. Indeed, as a very competent embryo-logist (Mauritzon, 1939) recently remarked, the resemblances and differences in the embryological characters of the members of a family are sometimes of such a fine type, that they can neither be brought out in words nor even in a drawing but can only be appreciated under the microscope. He nevertheless considers them to be of distinct value in delimiting the smaller groups and in determining their interrelationships with one another.

Let us now take some specific instances where embryology has rendered an important service in the determination of the proper position of some difficult families or in giving a new orientation to our ideas of their affinities.

The relationships of the family *Empetraceæ* exercised the minds of systematists for a long time and it has been placed by some authorities in the Monochlamydeæ, and by others in the Sapindales or the Celastrales. Samuelsson's work (1913) has definitely shown however that its proper place is with the Bicornes, a group which is characterised by the following well-marked embryological features :—

1. Absence of a fibrous layer in the anthers.

2. Presence of a glandular tapetum which does not become amœboid.

3. Pollen grains remaining together in tetrads.

4. Ovule with a single integument and a thin ephemeral nucellus which completely disappears in later stages so that the embryo-sac lies in direct contact with the integumentary tapetum.

5. Absence of parietal cells in the ovule, the hypodermal arches-porial cell functioning directly as the megaspore mother-cell.

6. Embryo-sac of the monosporic eight-nucleate type with small ephemeral antipodals.

7. A hollow and fluted style which connects the lumen of the ovary with the outside and along which the pollen tubes make their way into the ovary.

8. Endosperm cellular, the first two divisions being transverse and giving rise to a row of four cells placed above one another.

9. The formation of endosperm haustoria at both ends of the embryo-sac, micropylar as well as chalazal.

10. A single-layered seed-coat formed from the outermost layer of the integument, the remaining layers becoming absorbed during the growth of the embryo-sac and embryo.

All these are perfectly standard stages in Erican embryology, a combination of which is not known to occur in any other order except the Bicornes. The Empetraceæ show a close correspondence in all respects, while the Sapindales and Celastrales differ from them (Bicornes) in so many ways that Samuelsson may be said to have established his point of view fully and completely. Hutchinson's assignment of the Empetraceæ to the Celastrales is therefore considered

by Schnarf (1933, p. 283) to be due to nothing but a "ganz besonderer Verständnislosigkeit ".

On the other hand, the Lennoaceæ, which have sometimes been placed in the Bicornes (Hutchinson, 1926) certainly do not belong here. In the very first place, the equality in number of their stamens and corolla-lobes (contrasted with the obdiplostemony of the Ericales), the alternation of the parts, the adnation of the filaments to the corolla and the dehiscence of the anthers by longitudinal slits, form a weighty objection against this view. Add to these the fact that the Lennoaceæ have a short and solid style, a normally developed endothecium, pollen grains separate from each other, and a seed-coat which is more than one-layered. Svensson (1923) and Copeland (1935) therefore correctly consider the assignment of the Lennoaceæ to the Bicornes as quite untenable on embryological as well as other grounds and suggest that they might more reasonably be placed among the Tubiflorales as a separate suborder occupying a primitive position.

Let us now pass on to another group, the Cactaceæ. F. Vaupel (1925), in the latest edition of *Engler-Prantl's Pflanzenfamilien*, writes that there is hardly a family in the plant kingdom, the allocation of which has allowed so much scope to individual tastes as this. Wettstein placed it in the Centrospermales and Engler-Prantl in a separate order Opuntiales near the Passifloraceæ. Hutchinson has erected the order Cactales and placed it closest to the Cucurbitales.

The views of the great Viennese systematist have received very definite confirmation in this respect from the works of two embryologists, Mauritzon (1934) and Neumann (1935). Although additional work on this family would be welcome, the following features seem to be well established :—

1. A secretory tapetum of parietal origin.

2. Division of pollen mother-cells of simultaneous type.

3. Pollen grains tri-nucleate at the time of shedding.

4. Ovules campylotropous with strongly curved and massive nucelli.

5. Two integuments ; and the swollen lips of the inner, which alone forms the micropyle, protruding out to approach the funiculus.

6. A hypodermal archesporial cell which cuts off a wall cell.

7. A nucellar cap formed by periclinal divisions of the cells of the nucellar epidermis.

8. Embryo-sac of the Normal type.

Several other characters, for which a reference may be made to Frl. Neumann's original paper, point to the conclusion that the Cactaceæ belong to the Centrospermales and form a sort of bridge between the Aizoaceæ and Portulacaceæ. An interesting point, which has probably been overlooked by several workers but is nevertheless of considerable importance is the presence, in the chalazal part of the ovule, of a "Hohlraum" or "Luftspalt" between the two integuments and sometimes also between the inner integument and the nucellus.

This is quite distinctive of a number of other families belonging to the Centrospermales and its occurrence in the Cactaceæ is therefore of great significance.

On the other hand, a comparison of the embryology of the Cactaceæ with that of the Passifloraceæ offers so little by way of resemblance that any close relationship between them appears to be most unlikely.

.Take again the Onagraceæ, in which the genus *Trapa* has long been considered to occupy a somewhat anomalous position. All the plants of this family so far investigated show a monosporic four-nucleate embryo-sac, *Trapa* alone being an exception with an eight-nucleate embryo-sac and a well-developed suspensor haustorium. From the embryologist's standpoint this strongly supports the case for a removal of this genus to a separate family—a course which has now been adopted by some systematists by erecting the family Hydrocaryaceæ for its reception. Regarding the relationships of the Onagraceæ with other families of the order Myrtales, it seems very likely that it has been derived from the Lythraceæ (Tischler, 1917) in which the ephemeral antipodals show the way to a complete omission of the chalazal part of the embryo-sac, leading to the four-nucleate condition of the Onagraceæ. I understand that this view is not challenged by systematists.

In Prof. Schnarf's laboratory at Vienna, some very important work has been done on the embryology of the Liliaceæ and Amaryllidaceæ, which has a great bearing on the interrelationships of the various sub-families and tribes included under these large and difficult families.

Taking the sub-family Lilioideæ, Engler (1888) divided it into the tribes Tulipeæ and Scilleæ. Schnarf (1929) stressed the sharp embryological differences between them, and in the second edition of the "Natürlichen Pflanzenfamilien" Engler and Prantl (1931) removed the Scilleæ from the association giving it the status of an independent sub-family, the Scilloideæ, so that the name Lilioideæ is now synonymous with the former Tulipeæ. Frl. Rosalie Wunderlich, a pupil of Prof. Schnarf, has again (1937) stressed the great contrast between the two (see taole below) and even hinted at the desirability of separating them into two distinct families. She rightly points out that in more than one respect the Lilioideæ appear to be a derived group while the Scilloideæ are more primitive ; the latter should therefore be placed *before* the former and not after as it has been done by Engler and Prantl.

Dr. Wunderlich further adds that the Scilloideæ themselves fall into two tribes : one with the Helobiales type of endosperm (*Ornithogalum, Muscari* and *Puschkinea*) which she calls the *Ornithogalum* group and the other with the *Nuclear* type including the genera *Hyacinthus, Scilla, Camassia* and *Galtonia*.

Scilloideæ	*Lilioideæ*
1. Parietal cell always present in ovule	Parietal cell absent
2. Embryo-sac of *Normal* or sometimes *Allium* type	Embryo-sac of *Fritillaria* type

Scilloideæ	*Lilioideæ*
3. Endosperm of *Nuclear* or *Helobiales* type	Endosperm of *Nuclear* type
4. Embryo large, occupying almost the entire length of the seed	Embryo small (*Tulipa, Lilium, Fritillaria, Erythronium,* etc.) and occupies only a small space in the seed
5. Generative cell small and slender, not easily stainable with acetocarmine	Generative cell large and broadly spindle-shaped, staining easily with acetocarmine
6. Male nuclei ± spherical	Male nuclei ± elongated
7. Chromosome no. variable	Chromosome no. usually 12
8. Raphides present	Raphides absent
9. Septal nectaries present	Septal nectaries absent

The systematic position of the Moringaceæ has long been a matter of some doubt. The astonishing observations of Rutgers (1923) on the embryo-sac and embryo of *Moringa oleifera* only increased this element of uncertainty. He reported in this plant the absence of a parietal cell in the ovule and the presence of a five-nucleate embryo-sac and a free nuclear embryo. My pupil, Prof. V. Puri of Meerut (1940), has shown that a parietal cell is *present*, the embryo-sac is of the normal *eight-nucleate* type and what Rutgers considered to be a free nuclear embryo is merely a group of some *endosperm nuclei* at the micropylar end of the sac, the fertilised egg having escaped his notice altogether! The resemblances which the Moringaceæ show to the Capparidaceæ in embryology and carpel morphology make it seem fairly certain that their correct position is in the order Rhœadales and the place assigned to this family by Hutchinson—between the Capparidaceæ and Tovaricceæ—is therefore justified.

Although provisionally placed with the Rosales, there has always been some doubt regarding the interrelationships of the Podostomaceæ with the other families of this order. The extensive work on the embryology of the Crassulaceæ and Saxifragaceæ done a few years ago by Mauritzon (1933) has however brought out certain features which make it almost certain that the Podostomaceæ are much reduced apetalous derivatives of the Crassulaceæ. The peculiar structure of the ovules of the former appears to be brought about merely as the result of a continuation of the reduction already seen in the Crassulaceæ and *Crassula aquatica*, in particular, whose mode of life is somewhat similar to that of the Podostomaceæ and which has the most reduced endosperm in the Crassulaceæ, may well form a transitional stage leading to the complete suppression of this tissue in the Podostomaceæ. A striking agreement between the two families is the presence of a highly developed suspensor haustorium, a feature which in Mauritzon's (1939, p. 38) opinion offers "such an eloquent proof" of their relationship "as to convert many doubters",

It is possible to add numerous other instances where embryology has 'rendered signal service to systematic botany, but considerations of time and space forbid me from citing them here. I believe that a stage has now arrived when we should try to have an embryological formula for each family as a supplement to the well-known floral formula so commonly used by systematists. To make my meaning clear I give below the embryological formula of the family Alismaceæ with which I have been particularly familiar as the result of the work done on it by my pupil Dr. B. M. Johri of Agra and later by Balwant Singh and myself (Maheshwari and Singh, 1943) at Dacca :—

ANTH.-TAP. (amœboid) ; DIV. OF P.M.C. (succ.) ; P. (3-nucl.) ; OVULE (2-integ. ; anat.) ; PAR. CELL (absent) ; E.S. (*Allium* T.) ; END. (*He.* or *Nu.* T.) ; EMB. (*Sag.* T.).

Put in plain English this means that the anther tapetum is of the amœboid type ; the divisions of the pollen mother-cells are successive ; the pollen grains are 3-nucleate at the time of shedding ; the ovule is anatropous and has two integuments ; no wall cell is cut off by the primary archesporial cell which functions directly as the megaspore mother-cell ; the embryo-sac development is of the *Allium* type ; the endosperm is of the *Nuclear* or *Helobiales* type ; and the embryo is of the *Sagittaria* type.

It may be possible by means of further abbreviations to include this information in a still shorter space and to make other improvements so as to devise a symbolisation which will be internationally acceptable. I suggest that this point may be discussed, by those who are interested, in the pages of one of our monthly journals like *Current Science*.

The embryologist would however be glad to admit that he lays no claim to erect a phylogenetic scheme of his own. Indeed there are some very definite limitations to the embryological method, for, owing to parallel, convergent and regressive evolution, similar embryological characters may often be found in widely separated groups and if a system of classification were to be set up on such considerations alone, some rather fantastic results are bound to ensue. But, with the main lines of phylogenetic classification already chalked out by the systematist, it is possible for the embryologist, the cytologist and the anatomist to use this as a background and to help him in making it more perfect. A natural system has to be discovered (for it is already there) and not invented. In order to do this we have to do real detective work and take the aid of every branch of botany. Once a group has been assigned to its true place, every character that is studied will only serve to strengthen its position. On the other hand, if there are any discrepancies, they will be brought out in a more glaring fashion by the study of its internal structures (as these are less influenced by the environment) than the external.

I understand that in some of the big herbaria of the U.S.A. steps are being taken to have along with the dried specimen a preparation or two showing the structural features of its wood. This is a laudable effort, but it ought to be extended still further so as to include

in each case about half a dozen preparations of the pollen, ovule and seed as well.

APPLIED AND EXPERIMENTAL EMBRYOLOGY

Now we come to Applied Embryology which has evolved, for the most part, only during the last two decades or so. It is hardly possible to do any justice here to this subject, for although still in its infancy, it already has such a voluminous literature as to defy any attempt to review it in a few pages. I shall therefore satisfy myself merely by indicating some of the main lines on which work is being carried on in this field.

Before any improvement of our crop plants can be undertaken through breeding methods, it is necessary to have *in each case* a thorough understanding of the behaviour of the flower throughout its development and the setting of the fruit. Of the greatest importance in this connection are :—a study of the viability of the pollen and the optimum conditions for its storage and germination ; the receptivity of the stigma ; the rate of pollen tube growth under different conditions of temperature and humidity ; the interval between pollination and fertilisation and how it can be influenced by external conditions ; and the quantity of pollen necessary for a proper fruit set. Considerable work of this nature is being done in America and Russia, regarding the immense value of which in our breeding programmes there can be no question.

It is said that the Arabs put aside the pollen of the date palm from year to year so as to ensure a supply of dates even in the possible event of the male flowers failing to develop or the female flowers developing precociously. If we succeed in devising suitable conditions of temperature and humidity for storing the pollen of other cultivated plants, which normally is not so long-lived, we may be able to cross two varieties which flower on widely different dates or which are separated by considerable distances from each other. In the latter case it may be possible to transport the pollen by air from one place to another. We may hopefully envisage the possibility of opening one or more "pollen banks" in each country, where pollen of almost every important plant of economic value will be stored under optimum conditions and supplied to recognised workers, gratis or on a moderate charge.

Sterility and unfruitfulness are often caused by a very slow growth of the pollen tube. The flowers do not remain attached to the plant for an indefinite period and unless fertilisation takes place within a reasonable time, varying with the species under consideration, abscission takes place at the base of the style and fruit setting is consequently prevented. Premature as well as delayed pollination have the same result and we therefore need full information regarding all of our fruit trees and crop plants on the rate of pollen tube growth and the time when the stigma is most receptive. The optimum conditions for the germination of pollen also need to be investigated more fully. Tischler's (1910) discovery that much of the pollen of certain species of *Cassia* occurring at Buitenzorg fails to develop without an

outside supply of diastase illustrates the need of such work from various points of view.

Another aspect of applied embryology is a study of the possibility of obtaining a fruit set without the generally associated seed formation. There are a number of our edible fruits where the pericarp is the chief edible portion and the presence of seeds is neither necessary nor desirable. It was found possible in several cases to do away with the fertilisation of the egg cell and give the ovary the necessary stimulus for further development by the application of pollen extracts. This led to a chemical analysis of the latter which in turn opened the way for the induction of artificial parthenocarpy through the use of growth hormones (for literature see Maheshwari, 1940 ; Gustafson, 1942).

A very important paper was published in 1928 by C. A. Jørgensen, which showed the way to the induction of parthenogenesis in flowering plants. He pollinated the stigmas of *Solanum nigrum* with pollen from some other species of this genus. Most of the fruits were seedless but a few were found to have formed 2 to 8 seeds which gave rise to haploid plants of *Solanum nigrum*. An embryological study showed that in certain cases the foreign pollen had germinated successfully and the pollen tubes had also reached the embryo-sacs, but the male nucleus which enters the egg cell eventually disintegrates and disappears. The egg cell thus develops by itself into the embryo, stimulated no doubt by the entry of the male nucleus. The plants produced from the resulting seeds are therefore haploids resembling the maternal parent. In other cases, apparently, the male gamete alone may give rise to the embryo as inferred from the characters of the offspring which resembles the paternal parent but as far as I am aware the cytological and embryological processes leading to this condition are still unknown. Jørgensen's work opened the way towards the artificial production of haploids in a number of species and races. Although weak and valueless in themselves, they are of great utility in giving us an insight into the genetic constitution of the parent variety and for the production of homozygous diploids by a subsequent doubling of the chromosomes.

The effects of X-rays, colchicine treatment and exposure to extreme temperatures on the normal course of development are other aspects of recent cytoembryological research, which it is impossible to deal with here. It need only be said that while a fair amount of work has been done on the manner in which they influence ordinary mitotic and meiotic divisions, we are still very much in the dark about their effect on megasporogenesis, fertilisation and the development of other ovular structures like the endosperm and the embryo.*

THE PLACE OF EMBRYOLOGY IN BOTANICAL TEACHING

At the end of this very short and imperfect sketch of the aims and scope of embryological research we may now consider another aspect of the subject, *e.g.*, its place in botanical teaching. That a study of embryology demands a more thorough training in microtechnique

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* The important work, which is being done by Blakeslee and others on the artificial culture of excised embryos, will be reviewed elsewhere.

than is usually needed for other branches of plant science is a fact well known to everybody and it is perhaps for this reason that while much time is spent in our class-rooms on vegetative anatomy, in several universities little or nothing is shown to the students concerning angiosperm life-histories. A great opportunity is lost thereby of training them in the art of observation, reconstruction and critical interpretation. While granting that it is the advent of the microtome, as an instrument of precision in making serial sections, that has done so much for the recent progress in this science, it is not to be imagined that an elaborate scheme of fixing, dehydration, infiltration, imbedding, cutting, and staining is necessary in all cases. Much can be seen and shown by simpler methods. All stages of microsporogenesis and the maturation of pollen grains can be observed by making smears of suitable materials like *Tradescantia, Gloriosa,* etc., stained with either gentian violet or Feulgen (for technique see Darlington and La Cour, 1942). It is possible to mount the pollen grains of *Ottelia* and *Hydrilla* in acetocarmine and make a preparation, showing the vegetative and generative (or sperm) cells, even under the low power, in less than 5 minutes. Anthers of even herbarium specimens are often quite usable for such purposes (see Leitner, 1938). Some years ago Dr. Wulff and I (Maheshwari and Wulff, 1937) gave a schedule for making permanent mounts of pollen tubes to show the division of the generative cell and the organisation of the male cells. The common garden species of *Impatiens* is very good for this purpose as the pollen grains germinate readily and show the desired stages in only half an hour's time. Some of the Pontederiaceæ like *Monochoria* and perhaps several other plants may be equally suitable for the purpose. With the use of vital stains such slide cultures of pollen tubes may be used for studying the movement of the cytoplasm and the male gametes.

An observation of the stages in megasporogenesis and embryo-sac formation involves greater difficulties as the cells concerned are encased in several layers of other cells belonging to the nucellus and integuments, but Hillary (1940) has recently developed a technique by which he has been able to follow the development of the embryo-sac of *Lilium longiflorum* right from the megaspore mother cell up to the time of fertilisation and beyond, without cutting any sections. The ovules are here taken out from the ovary and the tissue around them removed as far as possible. Then they are fixed, washed, and stained with Feulgen's reagent in small vials or tubes. From the SO_2 water they are transferred to a drop of acetic acid placed on a slide and crushed under a coverslip. The author presents photomicrographs made from such preparations, which show the nuclei and chromosomes standing out quite distinctly in the colourless cytoplasm of the embryo-sac.

In his work on *Notonia grandiflora*, Ganesan (1939) used a somewhat similar method in order to select material of the right age for a study of the reduction divisions in the ovule. In this case an ovule is dissected out and mounted in a drop of acetocarmine mixed with an equal quantity of 1% safranin in 50% alcohol. By gentle and gradual increase of pressure on the coverglass, the nucellus is now freed from the

thick integument and in about half an hour's time the megaspore mother-cell nucleus is adequately stained for the purpose. By this method the author was able to exercise some judgment at the time of fixing the material and save much labour which would otherwise have been wasted if the cutting had been done at random. Poddubnaja-Arnoldi's (1938) "rapid method of embryological investigations" is essentially similar except that she recommends a mixture of acetocarmine and glycerine. She was thus able to follow the development in some plants upto the first stages in the formation of the embryo.

Suitable material for watching the process of fertilisation without recourse to section cutting has not yet been found* but one may try for this purpose such plants as *Torenia* and *Utricularia* in which the nucellus degenerates early and the upper part of the embryo-sac protrudes out of the micropyle so that it is naked and therefore more readily observable. The styles and stigmas of *Portulaca, Ottelia* or *Monochoria* should also be examined in order to follow the course .of the pollen tube from the stigma to the ovule. A treatment with lactophenol and cotton blue often facilitates such observation.

That certain interesting features of endosperm morphology can be brought out more clearly from whole mounts of suitably dissected material than from sections, is shown by the work of Kausik (1939) on *Grevillea*. Dr. Kausik discovered in this plant a curious "worm-like" structure, which he calls the "vermiform appendage", formed by the chalazal part of the endosperm. This was missed by earlier observers since they used only sections which naturally fail to give any complete or intelligible picture of this tortuous organ.

There is perhaps no way of studying the development of the embryo except by cutting thin sections of the ovule but favourable material may yet be discovered in which the technique so successfully used by Buchholz (1938) in the study of conifer embryogeny will be found adaptable for at least some stages of this process. Also, in certain cases the seed coat may be so transparent (B. G. L. Swamy tells me that this is the case in many orchids) that it is possible to see the embryo in whole mounts of the seed without recourse to sectioning.

Let me explain why I am so keen that students should cut sections and make whole mounts or try other methods so as to see the entire process of development of the gametophytes and embryo in angiosperms as an essential part of any course in botany. This is because there are few other spheres of botanical study which offer a similar variety of technical problems or opportunities for the development of a critical attitude which is the most important quality that a young worker must learn to imbibe. I hope to be excused for citing here the case of a student who, having just taken the Master's degree, placed before me with great satisfaction a set of slides in which he claimed to have seen "all" stages of the development of the embryo-sac— 1-nucleate, 2-nucleate, 3-nucleate, 4-nucleate, 5-nucleate, 6-nucleate,

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* *Monotropa*, which is said to be very favourable for this purpose, is unfortunately not available in this country except in the hills and other inaccessible places, and the plant is not amenable to cultivation owing to its saprophytic habit.

7-nucleate and 8-nucleate. When the preparations were scrutinised it was found that all the sections were of mature embryo-sacs, but as the nuclei were spread apart into several sections, they were counted as they came, some here and others there, and imagined to be *stages in development* of an embryo-sac. Again, scores of students get away through a university course with the impression, gained from a study of book figures, that the integuments are lateral processes developing from the right and left sides of the nucellus, although a cross-section of the ovule or a whole mount of the same would have easily convinced them that it is not so.

I should add that it is not merely the student who makes mistakes but that even the experienced researcher is liable to be misled into such erroneous interpretations as may in some cases ruin his reputation as a scientific worker. Indeed, there are so many pitfalls in the correct interpretation of the material that the embryologist must always remain as watchful and alert as the worker on fossils. As an instance may first be cited the case of the *Lilium* embryo-sac whose development was repeatedly and very intensively studied by the most competent workers like Strasburger, Coulter, Mottier, Guignard and others. And yet, all of them were mistaken as the excellent work of Bambacioni (1928) showed a few years later. The embryo-sac of *Plumbagella*, long considered to be the most reduced among angiosperms, has turned out to be but a modification of the type seen in *Fritillaria* and *Lilium* (Fagerlind, 1938 ; Boyes, 1939). *Plumbago* has also been shown to have a development very different from that originally described for it by Dahlgren (see Haupt, 1934) and this has now been confirmed for another member of the same family, *Vogelia indica*, found in Rajputana (Mathur and Khan, 1941). In *Euphorbia heterophylla*, Sanchez (1938) recently reported a tetrasporic embryo-sac which has on reinvestigation turned out to be of the normal monosporic type (Maheshwari, 1942). Then again, the embryo-sac of *Rudbeckia*, which Palm (1934) believed to be of an entirely new type has been found to correspond with the now well-known *Fritillaria* type (Maheshwari and Srinivasan, 1944).

Another kind of error which is of frequent occurrence is the mistaking of the integument for the nucellus or *vice versa*. Even so recently as 1938 Houk fell into such an error in the case of the ovule of *Coffea* and in his confusion stated that the tissue may be regarded as an "integument-nucellus". Joshi (1938), Mendes (1941) and several other workers have shown that the nucellus and integument are both formed normally but the former soon disappears as is usual in most Sympetalae. A similar mistake appears to have been made by Pannochia-Laj (1938) who writes that in *Lochnera rosea* the ovule is peculiar in that it is not possible here to delimit the nucellus from the integument. In another genus, *Fouquieria*, the structure which was supposed to be a " massive " nucellus is really the inner integument, the former being extremely reduced and ephemeral (see Khan, 1943). Evidently Dr. Woodcock (1943) has also been misled when he says that in *Ipomœa rubro-cœrulea* the ovule "has no distinct integument" and the micropyle is formed by an "invagination at the end of the ovule next to the funiculus (see Maheshwari, 1944b).

The origin of the haustorial processes in the ovule has been another fruitful source of errors and misinterpretations. To mention only two such cases, Heinricher (1931–32), in his monograph on the genus *Lathræa*, stated that the micropylar haustoria are formed from the synergids and the chalazal from the antipodal cells. This was promptly contradicted and disproved by Glišic' (1932) who made a thorough study of *Lathræa squamaria* and found that both the haustoria are formed from the endosperm. A similar mistake made by G. O. Cooper (1942), working on *Lobelia cardinalis*, has already been commented on by me a few months ago (Maheshwari, 1944*a*).

Without citing further instances, I shall conclude this portion of my address merely by saying that even if the laboratory work in this subject makes greater demands upon the energy and resourcefulness of the teacher, this should not be grudged, as through this the young pupil gets such a stimulus for his mental development as is sure to be of use to him ever afterwards in his future career.

THE FUTURE

And now we proceed to the future.

It is said by some that the days of descriptive embryology are now over. This is far from true in my opinion. We need more of such investigations and will continue to do so for a long time. What needs to be emphasized, however, is that the descriptions must be full and accurate and the interpretations checked as critically as possible with preparations of the highest quality. As I have mentioned earlier, results of greater value may be expected if attention is focussed on a comparative study of only one aspect of the life-history at a time, *viz.*, male gametophyte, ovule, embryo-sac, pollen tube, etc. Each of these requires the study of a vast amount of literature and sometimes a technique different from that used for the rest.

With respect to phylogenetic embryology there is a great scope in our country, for we have representatives of a number of families in India, Burma and Ceylon, which have either received little or no attention or which deserve more intensive study than has so far been bestowed upon them. I mention below the names of a few but the list is by no means exhaustive and is capable of amplification :—

Aristolochiaceæ	Loranthaceæ	Cyperaceæ
Balanophoraceæ	Magnoliaceæ	Dioscoreaceæ
Berberidaceæ	Myristicaceæ	Eriocaulaceæ
Burseraceæ	Myrsinaceæ	Flagellariaceæ
Callitrichaceæ	Nepenthaceæ	Hæmodoraceæ
Ceratophyllaceæ	Nymphæaceæ	Juncaceæ
Cornaceæ	Pittosporaceæ	Lemnaceæ
Crypteroniaceæ	Podostemaceæ	Marantaceæ
Dilleniaceæ	Salvadoraceæ	Najadaceæ
Dipterocarpaceæ	Simarubaceæ	Palmaceæ
Droseraceæ	Sterculiaceæ	Pandanaceæ
Ebenaceæ	Symplocaceæ	Stemonaceæ
Flacourtiaceæ	Thymelæaceæ	Triuridaceæ
Fumariaceæ	Zygophyllaceæ	Xyridaceæ
Hippocrateaceæ		Zingiberaceæ

A thorough investigation of so many families requires much time and patience and the participation of a band of workers properly trained in embryological methods. Fortunately we have a number of qualified embryologists at Mysore, Bangalore, Benares, Annamalainagar, Poona, Madras, Agra, Meerut, Calcutta and other places, and I venture to hope that, with the co-operation of some of our colleagues in Europe and America, we may be able to prepare in this country a new "Comparative Embryology of Angiosperms" written more or less on the lines of Schnarf's great work (now 15 years old) in which each worker will write an exhaustive and critical account of the embryology of the particular group with which he is most familiar as the result of his *own* researches, for the literature on the subject is now too vast to be surveyed in a satisfactory manner by any one person. Students of wood structure like Record, Bailey and Wetmore, of chromosomology like Tischler, and floral anatomy like Eames and Arber are working towards the same end for their particular subjects. I expect that some of us present here will live to see the day when few families of flowering plants will need to be assigned by guess work, and in any case, whether the end comes sooner or later, we must travel hopefully towards it.

One fact to be noted in this connection is that although the embryologist cuts sections of the flower at various stages of development, he frequently confines his attention to the development and organisation of the embryo-sac and the subsequent changes which take place *inside* it or to a study of the meiotic divisions in the pollen mother-cells, while the structure of the anther and ovary wall, the placentation, the integuments, the nucellus, and the chalaza are either dealt with in a more or less cursory fashion or not described at all. This is unfortunate as all of these yield characters of great systematic value. The structure of the seed and the fruit receives even less attention probably because of the difficulty of sectioning them, but a judicious use of dilute hydrofluoric acid can sufficiently soften them in many cases without causing any appreciable harm to the tissues.

Last of all we come to the comparatively new science of experimental embryology. This is a more difficult field, but one which is full of promise in so many ways. As some one once said, the plant breeder puts pollen on the stigma and 'prays' for results in the ovary! For a scientific explanation of his successes and failures and for finding out the ways and means of increasing the former and remedying the latter, he must turn to the cytologist and the embryologist. The work that has been started in recent years on the effect of X-rays, heat, chemicals, etc., on the artificial induction of mutations is still in its infancy and it opens up vast possibilities before us. Here the breeder, the cytologist, the embryologist and the physiologist, must all join hands so that not only do we get the maximum results from what we have but we may evolve new and still better varieties of plants and thus add to the health and happiness of the world.

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4

CHROMOSOMES OF *ERYTHRINA INDICA* LAMK.

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THE first observations on the chromosome numbers in the genus *Erythrina* L. (Fam. Papilionaceæ) were made by Tschechow and Kartaschowa, who reported for *Erythrina crista galli* L. [*Micropteryx crista galli* (L.) Walp.] in the same year in one of their papers (Tschechow and Kartaschowa, 1932a) *ca.* 40 and in another paper (Tschechow and Kartaschowa, 1932b) *ca.* 44 somatic chromosomes. The smallness of the chromosomes and their fairly large number in the root-tip cells might be the probable reasons for this obvious discrepancy. Next, Senn (1938) reported $2n = 42$ and $n = 21$ chromosomes in *Erythrina herbacea* L., and added that he too could not determine exactly the chromosome number in *E. crista galli*. The purpose of the present note is to report the chromosome number in the Indian Coral Tree, *Erythrina indica* Lamk., which grows wild along the Indian sea-coast and is widely planted in the gardens throughout the country for its large brilliant scarlet flowers. The materials for investigation, seeds and flowers, were obtained from trees growing at Guntur in the Province of Madras.

SOMATIC CHROMOSOMES

The somatic chromosomes were studied in root-tips obtained from germinating seeds. Most of the dividing nuclei showed 42 chromosomes (Fig. 1). These do not exhibit a wide range in size, but are small, slender objects, of nearly the same size and show either median or submedian attachment constriction. The somatic karyotype of *Erythrina indica* Lamk. thus appears to be identical with that of *E. herbacea* L. as sketched by Senn (1938).

During the examination of the sections of the root-tips, besides the monosomatic cells, some disomatic cells were also observed. The dividing nuclei of such cells showed 84 chromosomes (Fig. 2). Such cases of somatic doubling of chromosomes have been reported already in many Leguminosæ. Senn (1938) in his extensive work on the cytology of this family found tetraploid cells in *Albizzia Julibrissin* and *Cassia nictitans*, and Iyengar (1938) in *Cicer arietinum*, but the most comprehensive observations in this respect have been made by Wipf (1939) and Wipf and Cooper (1938 and 1940). They have reported the general occurrence of cells with tetraploid nuclei in the roots of several Leguminosæ, such as *Pisum sativum*, *Lathyrus latifolius*, *L. odoratus*, *Lespedeza tomentosa* and *Vicia villosa*, and find a definite
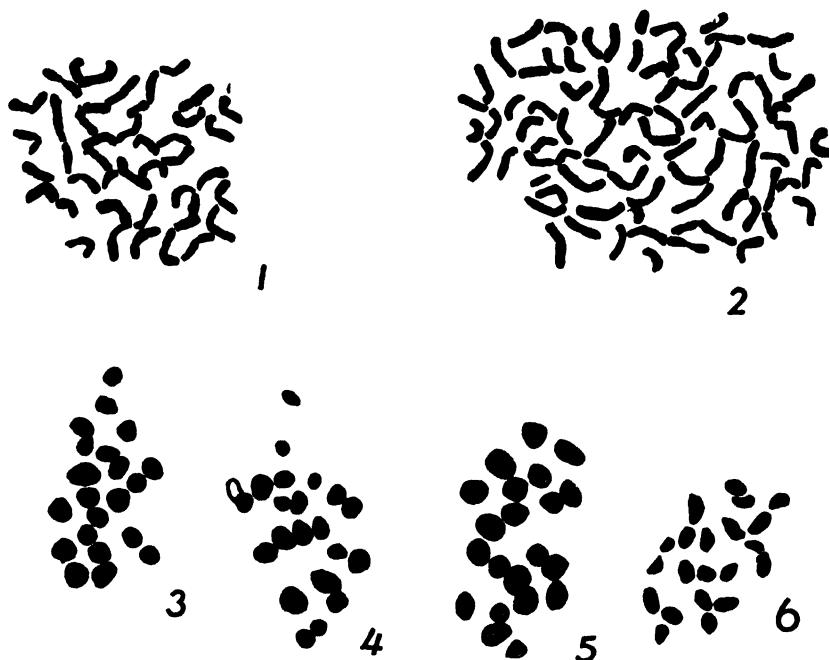
Figs. 1-6. *Erythrina indica* Lamk.—Fig. 1, Somatic metaphase showing 42 chromosomes. Fig. 2. Same showing a tetraploid nucleus with 84 chromosomes. Figs. 3–5. Polar views of Metaphase I ($n = 21$). Fig. 6. Metaphase II; only one plate of a P.M.C. is shown; $n = 21$. ×3,000.

relationship between the normal occurrence of disomatic cells in the roots of these plants and the formation of root nodules. The genetic significance of somatic doubling of chromosomes in restoring the fertility of sterile hybrids and in the origin of new species is already well known and need not be mentioned again here.

MEIOSIS

Observations on pollen mother cells undergoing meiosis showed $n = 21$ both at the I and II metaphase (Figs. 3–6). There are slight differences in size among the various bivalents at the I metaphase, but much importance need not be attached to this, as the size of bivalents in the polar views is determined by the presence and number of chiasmata (*cf.* Upcott, 1936). Polar views of meiotic chromosomes in *Erythrina indica* are also characterised by a marked degree of secondary association (Figs. 3 and 4). Groups of 2, 3 and 4 bivalents are quite common during the I metaphase and secondary association persists even in II metaphase.

DISCUSSION

The following table summarises the chromosome numbers reported so far in the genus *Erythrina* L.:—

Chromosome Numbers in Erythrina L.

Species	n	2 n	Author
E. crista galli L.		*ca.* 40	Tschcchow and Karlaschowa (1932*a*)
Do.		*ca* 44	Do. (1932*b*)
E. herbacea L.	21	42	Senn (1938)
E. indica Lamk.	21	42	This paper

The occurrence of $n = 21$ and $2n = 42$ chromosomes both in *E. herbacea* and *E. indica* make it very probable that in *E. crista galli* also there are 42 somatic chromosomes.

The occurrence of a rather high chromosome number in species of *Erythrina* as compared with most of the Papilionaceæ and secondary association both during the I and II meiotic divisions suggests that the genus *Erythrina* is of a polyploid nature. Taking into account that $n = 21$ is an unusual chromosome number in the Papilionaceæ, Senn (1938) remarked that this may indicate an ancestry through a 7 series or be the result of hybridisation from $n = 10$ and $n = 11$ ancestors with subsequent amphidiploidy. The latter suggestion, according to him, appears possible in view of the taxonomic position of the genus between forms with a basic number 10 and forms with a basic number 11. Future investigators of the cytology of *Erythrina* have to study this problem.

In the end, the author desires to express his appreciation to Dr. A. C. Joshi for his kind interest in the investigation and help in the preparation of this note.

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EMBRYOLOGICAL STUDIES IN THE THYMELÆACEÆ

I.　*Thymelaea arvensis* Lamk.

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THYMELÆACEÆ, by itself a very natural group, shows no close affinities with other families of flowering plants. It has been investigated by embryologists from time to time, but these investigations are mainly concerned with the development of pollen, ovule and embryo-sac. A few observations have been made also on the development of the embryo, but these accounts are very fragmentary and do not give either a correct or a complete picture of embryo development in any species. This has led the author to take up the present work.

PREVIOUS WORK

Broniart (1826) was the earliest botanist to pay attention to the embryology of Thymelæaceæ. He recognised the conducting function of the tissue arising at the base of the style (now called the obturator). Next, Hofmeister (1849) studied *Daphne laureola*, in which he observed a normal type of embryo-sac development. He also recorded that the mature embryo-sac was already formed before the onset of winter. Beauregard (1877) investigated the structure and development of the fruit in *Daphne*. Capus (1878), in his studies on the anatomy of conducting tissue in the angiosperms, drew attention to the formation of a bundle of tissue traversing from the base of the style to the micropyle. Vesque (1879) made some observations on *Daphne mezereum*. According to him, the archesporium in the ovule consists of a single cell and the embryo-sac development follows the normal type. The formation of nucellar cap and obturator was also studied by him. Prohaska (1883) studied the development of the embryo-sac and the endosperm in *Daphne*. He reported the occurrence of many antipodals in *Daphne Blagayana*. Schimper (1885) is referred to by Schnarf (1929)

as having reported the occurrence of plastids in the egg of the above species.

Strasburger (1884, 1885, 1909 and 1910) studied the embryology of *Daphne, Gnidia* and *Wikstrœmia* and particularly the problem of parthenogenesis in *Wikstrœmia indica.* He noted unicellular arches-porium in the ovule, a normal type of embryo-sac development, many antipodals in the embryo-sac of *Daphne Blagayana* and only three in *Daphne alpina* and *Wikstrœmia indica,* and the formation of the nucellar cap in the ovule. The development of the obturator and the endosperm in *Daphne* was also studied by him. He 'also made some observations on the structure and development of the anther and pollen. He states that the tapetal nuclei divide mitotically in *Wikstrœmia indica.*

Winkler (1904, 1906) made an embryological study of *Wikstrœmia indica* and investigated the problem of parthenogenesis in it. He traced the structure and development of the anther and pollen. According to him, the tapetum is formed by the innermost wall layer of the anther and its cells contained 2–6 nuclei. He described a normal structure in the embryo-sac. He studied the formation of obturator and pointed out that it formed a plug in the micropyle in *Wikstrœmia indica.* He also made some observations on the development of the endosperm and embryo.

Osawa (1913) investigated the development of pollen and embryo-sac of *Daphne* with special reference to sterility in *D. odora.* According to him, the division in the pollenmother cell is simultaneous. He reported the 3-nucleate condition of the mature pollen, and also some irregularities in the pollen formation in *D. odora,* besides degenerations in pollen and embryo-sacs. According to him, a linear tetrad of megaspores is usually formed in *D. odora,* but he also observed occasional formation of a T-shaped tetrad. Normally the chalazal megaspore is the functional one. He, however, found a few exceptional cases in *D. odora,* where a megaspore other than the chalazal one of the tetrad was functional. He noted 3–6 antipodals in the embryo-sac in *D. odora* and 30 or more in *D. pseudo-mezereum* and *D. koiusiana.*

Guèrin (1913, 1915) made a comprehensive study of the structure and development of the ovule and seed in Thymelæaceæ. He found vascular strands in the nucellus of *Dicranolepis, Craterosiphon* and *Synaptolepis.* His observations on the structure and development of the embryo-sac agree with those made earlier by others. He reported the occurrence of two cases of bilateral tetrads of megaspores in *Daphnopsis Sohwartzii.* Guérin also reported more than three antipodals in *D. Schwartzii* and many in *Thymelæa passerina* and *Dirca palustris.* He made some observations on the endosperm. Structure of the seed-coat was also described.

Dahlgren (1915) in his studies on the development of pollen, ovule and seed in angiosperms, mentions the formation of normal type of embryo-sac in *D. mezereum* before the advent of winter. Yamaha

(1926), in his comprehensive study of cytokinesis in the formation of pollen tetrads in the various plant groups, states that the cytokinesis in *Daphne* takes place by furrowing and passes through very rapidly. He also refers to the formation of a transitory cell plate after the heterotypic division in the pollen mother cells. Joshi (1937) noted 3-nucleate condition in the mature pollen of *Wikstrœmia indica, Thymelœa arvensis* and *Daphne mezereum*.

Fuchs (1938) gave a detailed account of the embryology of *Daphne odora* and made some observations on the embryology of *D. cneorum* and *Passerina pectinata*. She made a comparative study of the structure of pollen in *Daphne odora, D. cneorum, D. Blagayana, D. mezereum, Passernia pectinata, P. filiformis, Pimelea decussata, Pimelea ligustrina* and *P. spectabilis*. In all these she finds the mature pollen to be 3-nucleate and sperm cells to be elongated in the form of spirally twisted bands. She also describes the presence of rods and spines as structural features of the exine. The pollen has many germ pores. In *D. laureola, D. cneorum* and *Passerina pectinata*, she notes the formation of a nucellar cap, the presence of a plate-shaped tissue in the chalaza of the ovule and a conducting strand of elongated cells connecting it with the antipodal end of the embryo-sac. She also gives an account of the structure of the obturator. The embryo-sac development in the species studied follows the normal type. She reports the occurrence of linear, T-shaped and bilateral tetrads of megaspores. She finds 30–40 antipodals in the embryo-sac of *Daphne laureola, D. cneorum* and *Passerina pectinata*. According to her, these persist as dark points in ripe seeds. She states that polar nuclei lie close together near about the chalazal end and probably fuse just before fertilisation. The same was observed earlier by Winkler (1906), Strasburger (1909) and Guérin (1915). She followed the endosperm development in *D. laureola*. The fusion nucleus, according to her, divides before the fertilised egg. In the later stages cell formation was also noted by her. She also made a few observations on the embryo development in *D. laureola* and described the seed-coat structure.

Mauritzon (1939) described the structure and development of the ovule and seed in *Phaleria capitata*. He observed a remarkably extensive growth of the chalazal part in the ovule. He also made a few observations on the development of the pollen and embryo-sac in the same species.

Kausik (1940) published an account of the structure and development of the embryo-sac in *Lasiosiphon eriocephalus*. He described the structure of the anther and traced the origin of the tapetum. He noted the formation of a nucellar cap and conducting strand in the nucellus below the antipodal end of the embryo-sac. The development of the embryo-sac is normal. He noted the occasional presence of a spherical body in the synergids. According to him, the endosperm is of the free nuclear type. In the early stages the endosperm nuclei show a paired arrangement. He described a dense mass of cytoplasm in the chalazal part of the embryo-sac and states that the endosperm nuclei are large in this region and lie embedded in the plasma accumulated there.

He also made a few observations on the embryo. A 3-celled proembryo is formed. Further development is not followed in detail, but he states that a spherical embryo is formed after a few divisions. He also described the seed-coat structure, which is in agreement with the earlier accounts.

MATERIAL AND METHODS

The present paper deals with the development of the anther, pollen, ovule, embryo-sac, endosperm, embryo and seed in *Thymelæa arvensis* Lamk. This species grows in the upper Punjab, Kashmir, N.W.F.P., and extends from Afghanistan westwards to France and North Africa.

The material used in this investigation was very kindly placed at my disposal by Dr. A. C. Joshi of the Benares Hindu University along with a few prepared slides. It was collected from plants growing at Sopore (Kashmir), in the month of June 1938 and was fixed in formalin-acetic-alcohol. The customary methods of dehydration and infiltration were followed. Sections were cut $10–14\,\mu$ in thickness and were stained with Heidenhain's iron-alum-hæmatoxylin. Tuan's method of destaining with picric acid was followed.

ORGANOGENY OF THE FLOWER

The flowers arise singly in the axil of leaves. The floral parts arise in acropetal succession, the perianth making its appearance first, followed by the stamens and, last of all, the gynœcium (Figs. 14 *a, b* and *c*). The single whorl of perianth is urceolate and its 4 lobes are imbricate in bud. The upper part of the perianth shrivels up in the fruiting stage and the whole perianth remains as a membranous covering round the ovoid fruit.

The stamens are 8 in number (in two whorls) and are adnate to the perianth. The ovary is raised on a short stalk and contains a laterally attached single ovule. The style is short and ends in a stigma the surface cells of which form a number of papillæ. A prominent annular disc-scale develops round the base of the ovary.

DEVELOPMENT OF THE ANTHER AND POLLEN

The primary archesporium in the anther consists of only one row of cells in each of the four anther-lobes (Figs. 1 and 2). Soon after their differentiation the primary archesporial cells undergo a periclinal division, forming a layer of primary parietal cells towards the outside and a layer of primary sporogenous cells towards the inside (Fig. 3). The parietal layer of cells again undergoes another periclinal division and forms two layers of wall cells below the epidermis (Fig. 4). The inner of these only divides further into two layers, thus ultimately forming three wall layers below the epidermis (Fig. 5). The layer of wall cells immediately beneath the epidermis forms the endothecium, while the one immediately outside the sporogenous cells forms the tapetum. It is of the secretory type.

The tapetal nuclei divide mitotically and the tapetal cells become two-nucleate about the time when the pollen mother cell nuclei are in

the prophase of I meiotic division. The two nuclei lie closely appressed to each other. In the final stages the tapetal cell nuclei are found to contain up to 4 nucleoli. The tapetal cells, before degeneration, become filled with very small vacuoles.

In the mature anther, due to the growth of the pollen mother cells and the tapetum, the wall layer between the endothecium and the tapetum gets crushed. With the further growth of the anther, the epidermis gets very much stretched, the endothecium further enlarges and the tapetum slowly degenerates. In very mature anthers the epidermis is so thinned out that it is hardly perceptible as a separate layer at some places (Fig. 6).

Usually the primary sporogenous cells enlarge and form the pollen mother cells. However, quite frequently, a cell or two of the primary sporogenous layer may divide once before forming pollen mother cells. The pollen mother cell nuclei undergo two meiotic divisions and form pollen tetrads ultimately. During meiosis they do not round off but remain packed together within the mother cell walls. The pollen mother cells divide simultaneously. The orientation of spindles at the II meiotic division has been found to be at right angles to each other, but sometimes parallel arrangement is also seen. This results in the formation of both tetrahedral as well as bilateral pollen tetrads. Cytokinesis appears to pass through rapidly and takes place by furrowing.

The exine and intine are formed before the pollen grain becomes two-nucleate. Fully formed uni-nucleate pollen grain has a large vacuole and the nucleus occupies a peripheral position. Usually, it is 1-nucleolate, but two nucleoli have been observed quite frequently (Fig. 7). The nucleus divides and forms two nuclei which show considerable difference in their size. The larger one is the vegetative nucleus and the smaller is the generative nucleus. The latter is organised into a definite lenticular cell separated from the vegetative one by a curved wall (Fig. 8). This wall, however, soon disappears, both the nuclei being left in the general cytoplasm of the pollen grain. The formation of such a lenticular cell separated from the vegetative one by an evanescent wall was also noted in some Thymelæaceæ by Fuchs (1938).

The generative nucleus divides into two male nuclei which finally form two elongated sperms. The pollen grain thus becomes 3-nucleate, which condition has been noted as characteristic feature of the pollen in Thymelæaceæ (Osawa, 1913 ; Joshi, 1937 ; Fuchs, 1938). The pollen grain shows a general increase in size and in mature condition gets filled with starch grains (Fig. 10). The sperms are elongated and spindle-shaped. The chromatin is unevenly distributed and the sperms look like spirally twisted bands. The same was also observed by Fuchs (1938) in *Daphne*. The tube nucleus is spherical in the early stages, but later it takes an irregular shape and stains very deeply as in Amarantaceæ (Kajale, 1940).

The exine and intine are formed in the uni-nucleate stage of the pollen grain. The former is much thicker than the intine. As seen

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in sections, it is composed of light and dark staining parts, the latter taking the form of radially arranged rods covered on all sides by the light staining portions of the exine (Figs. 7–10, 12 and 13). The exine protrudes a little outwards on the surface where the rod-like portions are situated giving rise to small spines. The latter are united
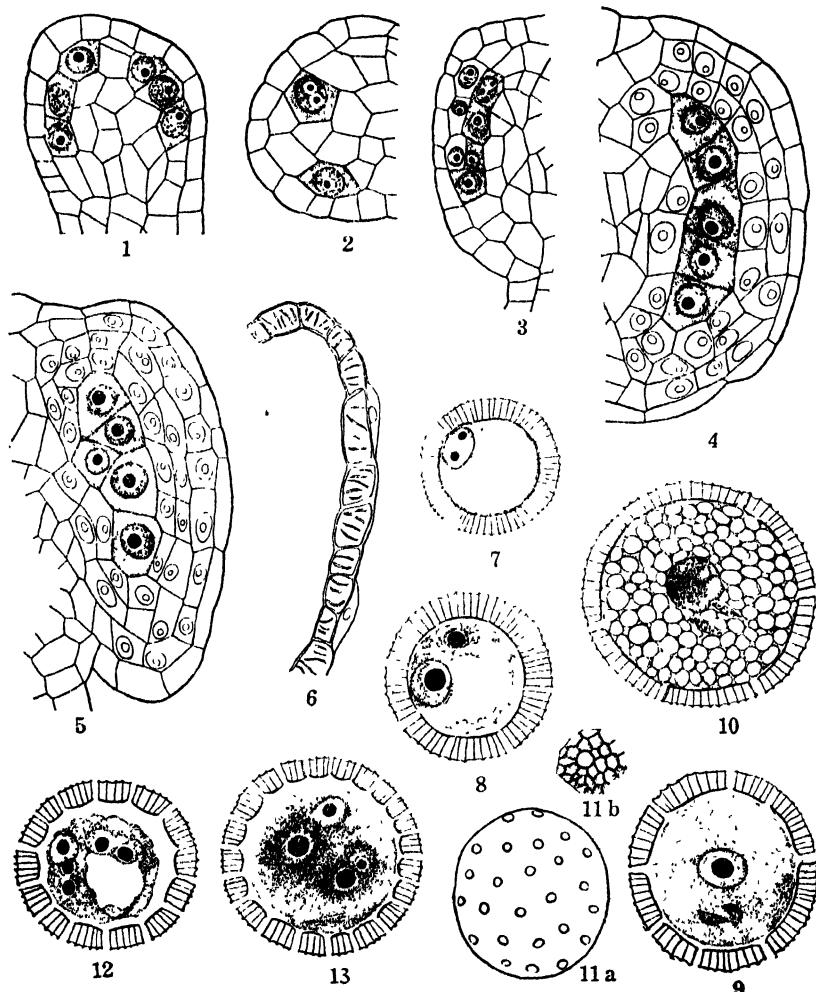
Figs. 1–13. *Thymelæa arvensis.*—Figs. 1–5. Various stages in the development of the anther. Fig. 2. Shows a transverse section, the rest represent longitudinal sections. Fig. 6. L.S. of wall of a mature anther. Figs. 7–10. Pollen grains in various stages of development. Fig. 11 *a*. Surface view of pollen grain showing germ pores. Fig. 11 *b*. A portion of exine showing a germ pore and sculpture on the surface. Figs. 12 and 13. Abnormal pollen grains showing extra nuclei. Figs. 1–5, ×546; Fig. 6, ×263; Figs. 7–11 *a* and 12–13, ×833; Fig. 11 *b*, ×3,360.

on the surface by ridges giving rise to a network like pattern on the outer surface of the exine (Fig. 11 *b*). The meshes of the network are usually 6-sided. There are many germ pores (about 50) and they are approximately equally spaced. They are usually circular in outline. Their arrangement on the exine is rather interesting. Usually, each pore, taken as centre, is surrounded by six other pores (Fig. 11 *a*). The pore membrane does not protrude out of the germ pores.

The mature pollen is sphæroidal in shape and the exine gets thinner on account of the stretching of the wall due to the growth of the protoplast of the pollen grain, very much like what has been described by Kajale (1940) in some Amarantaceæ. The diameter of the mature pollen grain approximately measures 35μ–37μ and that of the germ pore $1\cdot6\mu$–$1\cdot8\mu$. The distance between two germ pores (centre to centre) is about 5–6μ.

<div align="center">ABNORMAL POLLEN</div>

The pollen grains, as stated above, are usually 3-nucleate at the shedding stage. When the generative nucleus divides, it forms two sperm nuclei which are round in shape in the beginning. The vegetative nucleus is bigger in size and possesses a nucleolus. It stains less deeply than the generative nucleus with iron-alum-hæmatoxylin. The latter is distinguished from the vegetative nucleus by the absence of a distinct nucleolus, smaller size and deep staining capacity with iron-alum-hæmatoxylin. These features are also shared by the two sperm nuclei that arise from it.

Two exceptional cases of multi-nucleate pollen have been encountered in my preparations (Figs. 12 and 13). Fig. 12 shows a pollen grain with 5 nuclei, out of which one is larger in size. It shows a distinct nucleolus and stains less deeply than the rest with iron-alum-hæmatoxylin. The rest of the four are much smaller in size, take a deep stain and show no distinct nucleolus. From these features, it appears that the two sperm nuclei have undergone an extra division producing four daughter nuclei resembling the sperm nuclei in their general appearance. It may be mentioned here that four male nuclei (of about equal size), in pairs, have been observed by Dutt and Subba Rao (1933) in some pollen grains and in a pollen tube that has just reached the embryo-sac in the sugarcane (cross vellai ♀ ×B. 3412 ♂). Fig. 13 shows a pollen grain with four nuclei, out of which two are larger in size, stain less deeply and show a nucleolus, while the rest of the two resemble sperm nuclei. In this case, it appears that the extra nucleus has arisen due to the division of the vegetative nucleus.

Wulff and Maheshwari (1938) list a number of such abnormal cases of pollen having more than three nuclei in their review of the male gametophyte of angiosperms. The list includes *Lilium tigrinum* (Chamberlain, 1897), *Eicchornia crassipes* (Smith, 1898), *Sparganium simplex* (Campbell, 1899), *Yucca recurva* (Woycicki, 1911), *Cuscuta epithymum* (Federtschuk, 1931), *Atriplex hymenolytra* (Billings, 1934) and *Stelleria media* (Joshi, P. C., 1936). Bhargava (1936) reports

a 4-nucleate condition in many pollen grains of *Chenopodium album*. Juliano and Alcala (1935) reported the occurrence of 2–7 vegetative nuclei in *Musa errans* (Blance) Teodore var. *Botoan* Teodore.

Citing some of the above cases, Coulter and Chamberlain (1903) write, "In *L. tigrinum*, Chamberlain has often found a small cell cut
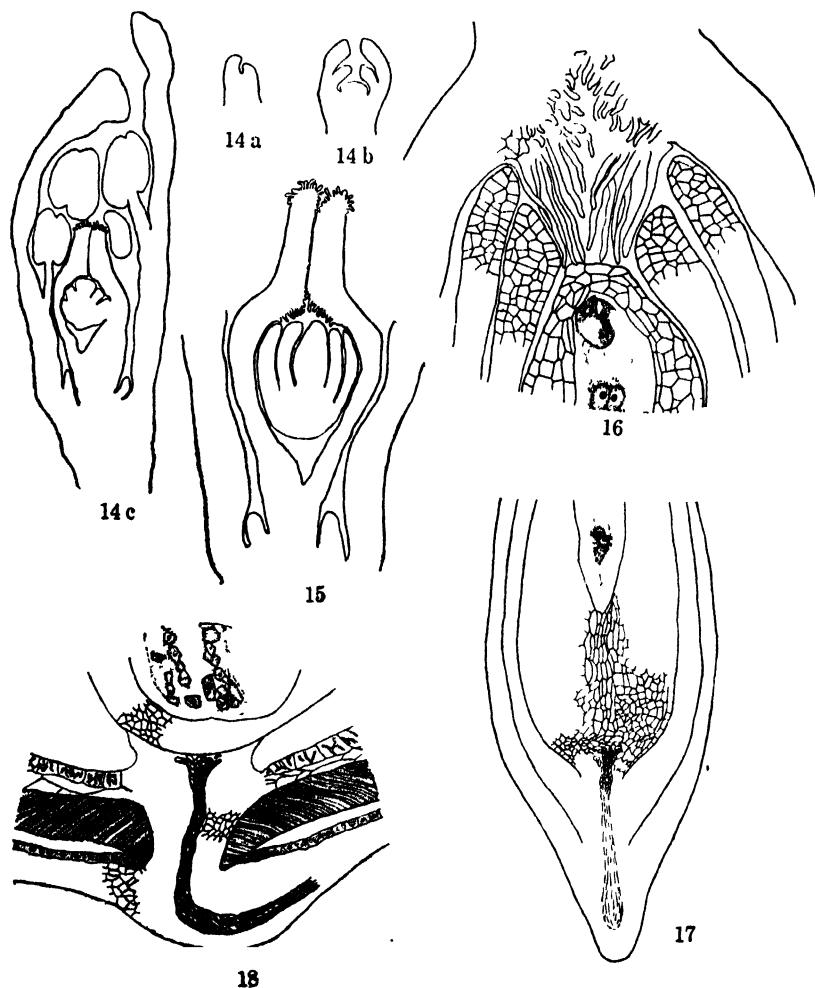
Figs. 14–18. *Thymelæa arvensis.*—Figs. 14 *a*, *b*, *c*. Stages in the development of the flower. Fig. 15. L.S. of the gynœcium showing the development of the obturator, ovule and the disc scale at the base of the stalked ovary. Fig. 16. L.S. base of the style and upper part of a mature ovule showing the obturator in the funnel-shaped micropyle. Fig. 17. L.S. of ovule (except the micropylar part) showing the conducting strand connecting the antipodal end of the embryo-sac with the ovular vascular trace. Fig. 18. L.S. of the chalazal end of an old ovule. Figs. 14 *a*, *b* and *c*, ×185; Fig. 15, ×78; Fig. 16, ×213; Figs. 17 and 18, ×126.

off by the microspore before the appearance of the tube and generative nuclei and the same cell was noted after the division of the generative nucleus. A similar cell was found by Smith in *Eicchornia crassipes* and by Campbell in *Sparganium simplex*. It is suggestive of true vegetative or prothallial cell, two of which so commonly occur in gymnosperms but the phenomenon is too unique as yet among the angiosperms to deserve more than a mention." According to Wulff and Maheshwari, Billings (1934) does not think it improbable that "an angiosperm would now and then be found exhibiting an atavistic tendency in producing a prothallial cell". P. C. Joshi (1936), however, thinks that there is no scope for interpreting the extra nucleus as a prothallial cell in the exceptional case of the 4-nucleate pollen grain recorded by him in *Stelleria media*. From the two cases recorded by the writer, it appears that the extra nuclei may be formed due to the further division of either the vegetative nucleus or the generative one.

DEVELOPMENT AND STRUCTURE OF THE OVULE AND EMBRYO-SAC

Ovule.—The ovary contains a single anatropous ovule. It is laterally attached, with the micropyle pointing upwards. First, it appears as a small hump. At about the time of division of the primary archesporial cell into an outer parietal cell and an inner megaspore mother cell, the integuments start as two annular rings one below the other. Ultimately the fully developed ovule assumes the anatropous form. In the early stages of development there is some space below the ovule (Figs. 14c and 15), but afterwards it is all occupied and the ovule touches the lower end of the ovary. There is no suggestion of the presence of a second ovule.

The ovule has two integuments. The micropyle, in fertilisable ovules, is funnel-shaped. It is formed by both the integuments, though the inner takes greater part at this stage. The micropyle receives the obturator. After fertilisation, the obturator dwindles gradually until, finally, it disappears in the seed. The integuments also grow and come close together forming a narrow micropyle in the seed. To start with the integuments are 3 cells in thickness, but in later stages the outer one becomes 4 cells in thickness and the inner 5 cells in thickness. Their various cell layers undergo different changes in the seed-coat. These are described later.

The nucellus is fairly massive. The nucellar epidermis, in the micropylar region, shows periclinal divisions and forms a nucellar cap 2–3 cells in thickness (Figs. 21, 22 and 23). In the mature ovules, the nucellus above the micropylar part of the embryo-sac is about 4–5 cells in thickness (including the cells of the nucellar cap) and, at the apex forms a slightly elongated protrusion. Usually it is about 4 cells in thickness on the sides of the embryo-sac and 15–20 cells in thickness below the antipodal end of the embryo-sac. In the chalazal region is developed a strand of elongated cells connecting the vascular bundle of the funicle and the antipodal end of the embryo-sac (Fig. 17). Such a conducting strand in the chalazal region of the ovule was also noted by Fuchs (1938) in some members of Thymelæaceæ and by

Kausik (1940) in *Lasiosiphon eriocephalus.* Guérin (1913) described the presence of vessels in the nucellus of *Dicranolepis, Craterosiphon* and *Synaptolepis.* Such vessels, however, are absent in *Thymelæa arvensis,* which, in this respect, resembles *D. laureola, D. cneorum, Passerina pectinata* (Fuchs, 1938) and *Lasiosiphon eriocephalus* (Kausik, 1940). A similar conducting strand in the ovule is also found in Lythraceæ (Joshi and Venkateswarlu, 1935 *a,* 1935 *b,* 1936 ; Venkateswarlu, 1937 *a*), in *Duabanga sonneratioides* (Venkateswarlu, 1937 *b*) and in Geissolomataceæ (Stephens, 1909).

Megasporogenesis and Embryo-sac.—The primary archesporium in the ovule consists of a single cell, which differentiates much before the integumental primordia appear (Fig. 19). It undergoes a periclinal division giving rise to an outer cover cell and an inner megaspore mother cell. Even before this division, the cells of the epidermis have divided once or twice periclinally to form the nucellar cap. The primary parietal cell undergoes a periclinal division and ultimately gives rise to two layers of parietal tissue under the nucellar cap. In *Daphne alpina* (Strasburger, 1909), *D. mezereum* (Vesque, 1879), *D. odora* (Osawa, 1913), *D. laureola, D. cneorum* and *Passernia pectinata* (Fuchs, 1938), a more extensive parietal tissue is formed making the megaspore mother cell deep-seated.

The megaspore mother cell forms a linear tetrad of megaspores, the chalazal-most of which is the functional one (Figs. 21–22). The three micropylar megaspores degenerate and they can be seen at the 4-nucleate stage of the embryo-sac (Fig. 23). No cases of T-shaped or bilateral tetrads of megaspores have been met with as in *D. alpina* (Strasburger, 1909), *D. odora* (Osawa, 1913), *D. Schwartzii* (Guérin, 1915) and *D. laureola* (Fuchs, 1938).

The development of the embryo-sac is according to the normal type (Figs. 21–27). The binucleate embryo-sac is characterised by the persistence of the chalazal vacuole. The same feature has been observed in *Lasiosiphon eriocephalus* (Kausik, 1940) and in Lythraceæ (Joshi and Venkateswarlu, 1935 *a,* 1935 *b,* 1936). In the 8-nucleate embryo-sac, the organization of the antipodals takes place slightly earlier than the egg-apparatus (Fig. 24). The two polar nuclei move towards the centre and meet about the middle of the embryo-sac. Usually the polar nuclei are 1-nucleolate, but in one case each of them has been found to be 2-nucleolate (Fig. 25). They fuse just before triple fusion. The two synergids, when fully developed, are hooked and have the usual chalazal vacuole. There is also seen a small vacuole in the apical region of the synergid (Fig. 28). The egg is usually situated a bit deeper than the synergids (Fig. 27) and has the usual flask-shaped form with a large vacuole above the nucleus (Fig. 29). In the early stages of the 8-nucleate embryo-sac, the antipodals are formed into three cells. Later, they multiply and form a large number of small cells (Figs. 27 and 31). Usually about 25–30 cells may be counted. During the endosperm formation, the antipodal end of the embryo-sac elongates and leaves behind the antipodal mass of cells on one side at about the middle of the embryo-sac (Figs. 32 and 33).

As can be seen from the review of previous work, many antipodals have been reported previously in *Daphne Blagayana, D. pseudo-meze-reum, D. koiusiana, D. laureola, D. cneorum, Passerina pectinata, Dirca palustris* and *Thymelæa passerina.*

The form of the embryo-sac varies at the various stages of its development (Figs. 24–29 and 30–34). Up to the time of fertilisation it is contained in the upper half of the nucellus, but later it elongates and extends throughout the length of the nucellus. With the growth of the embryo-sac the surrounding nucellar tissue is crushed.

An Abnormal Embryo-sac

As stated above, the embryo-sac in *Thymelæa arvensis* is formed according to the normal type. The mature embryo-sac contains very small antipodals in addition to the egg apparatus and the two polar nuclei. A single case of an abnormal embryo-sac with more than the usual number of nuclei has been met with. Fig. 29 shows the same. It is in an early stage of development and shows three antipodal cells and two polar nuclei lying together near the antipodal end, while in the micropylar end are seen two cells and five nuclei. Out of the two cells, one can be recognised as the egg judged from its form and structure. The second cell has a distinct vacuole in the basal part below its nucleus and seems to be the only synergid formed. Out of the five nuclei, three are found together in close association and indeed in the final stages of separation from each other. From their arrangement and association they, undoubtedly, seem to have arisen from the same nucleus in an amitotic manner. Out of the remaining two nuclei, the nucleolus of one is constricted and it seems to be on its way to divide amitotically. The situation seems to have arisen in the following manner :—First of all, as usual, two polar groups of four nuclei each should have been formed. Soon after, the antipodals are organised and the polar nuclei move towards the centre, meet each other and finally take their position together near the antipodal end. At about the same time, the egg cell and one synergid are differentiated at the micropylar end, out of the three nuclei left over there. The undiffer-entiated nucleus undergoes an amitotic division and then one of the two daughter nuclei, in its turn, follows suit. Thus, it ultimately leads to the formation of extra nuclei observed in the case recorded here.

Abnormalities in the structure of the embryo-sac have been previ-ously noted in other flowering plants. They may be grouped as below :

(i) *Embryo-sacs that show fewer than 8 nuclei.*—These arise either due to the suppression of one or more divisions or degeneration of a few of the 8-nuclei formed (usually in the chalazal pole of the embryo-sac). Instances of this kind are noted in good many plants and *Oenothera* (4-nucleate) type is supposed to be formed from a total suppression of development of the chalazal group.

(ii) *Embryo-sacs that show 8 nuclei but with abnormal organization of their constituents.*—The abnormal organization of the constituents may involve loss of polarity, reversed polarity, want of differentiation
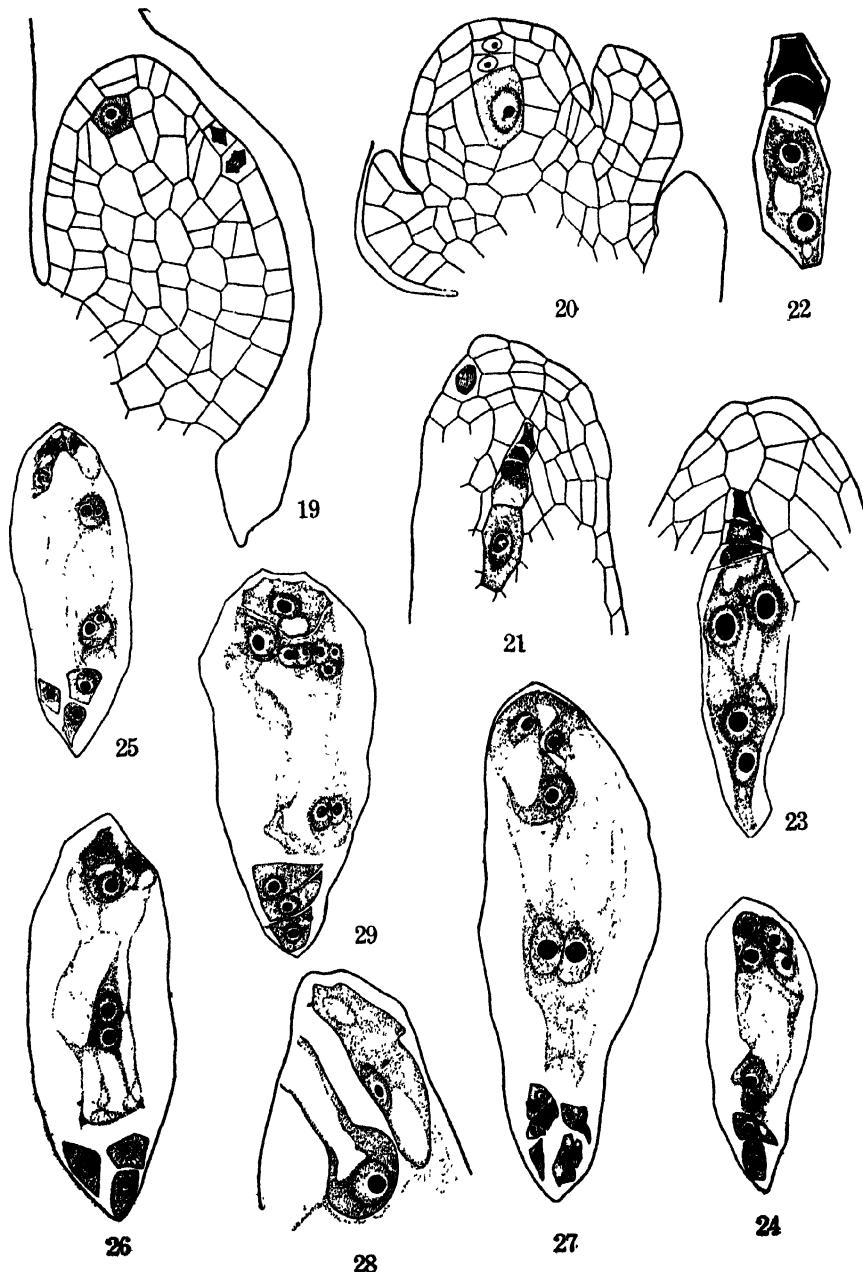
Figs. 19–29. *Thymelæa arvensis.*—Fig. 19. Primary archesporium. The epidermis of the ovule shows a periclinally divided cell. Fig. 20. M.M. cell

with two parietal cells above it. Fig. 21. L.S. of the nucellus of an ovule showing a linear tetrad of megaspores and nucellar cap formation. Fig. 22. 2-Nucleate embryo-sac. Figs. 23–27. Various stages in the embryo-sac development. Fig. 27. Shows a mature embryo-sac with many antipodals. Fig. 28. L.S. micropylar end of the embryo-sac showing the egg and a synergid. Fig. 29. An abnormal embryo-sac with extra nuclei. See text for further explanation. Figs. 19–21, 24–27 and 29, ×560 ; Figs. 22, 23 and 28, ×820.

in the constituents of the various groups, reduction in the number of members of one group and consequent increase in one or both the remaining groups, formation of extra egg cells at the expense of synergids, formation of extra number of synergids and eggs at the expense of other constituents, lack of differentiation in one or more constituents of either polar group, etc. Cases falling in this category are rare and always bear the character of abnormality.

(iii) *Embryo-sac with more than* 8 *nuclei.*—Extra nuclei arise due to secondary increase in the number of nuclei in either polar group. The extra nuclei so formed may add to the usual number constituting the egg-apparatus or polar nuclei or the antipodals. Secondary increase in the number of antipodals is found to occur as a normal feature in the embryo-sac of some plants, but secondary multiplication and increase in the micropylar group is very rare and always bears the character of abnormality.

The abnormal case of an embryo-sac in *Thymelæa arvensis* described above belongs to the last group. Schnarf (1929) and later Maheshwari (1941) enumerated important cases of abnormalities in the embryo-sac. Among these are included those that show extra nuclei. In 1880, Mellink recorded in *Luffa petiolata*, an embryo-sac divided into many cells and without any differentiation of either the antipodals or the egg-apparatus. In 1905, Shattuck reported occasional occurrence of super-numerary nuclei in some embryo-sacs of *Ulmus americana*, but recently, Fagerlind (1938), on a critical study of figures and statements published by various workers on *Ulmus* as well as his own preparations, suspects, that, in *Ulmus*, probably, a 16-nucleate embryo-sac is developed according to the *Drusa* form of the *Perperomia* type. In 1916, Dahlgren described abnormal cases of embryo-sacs in *Armeria alpina* and *A. plantaginea*. The embryo-sacs showed three synergids, one egg cell, three polar nuclei and five antipodals or four synergids, two egg cells, two polar nuclei and three antipodals. Next, Ekstrand (1918) recorded a few abnormal embryo-sacs in *Plantago major*. One of them showed seven cells in the egg apparatus, two polar nuclei and seven antipodals and another seven cells in the egg apparatus and three antipodals. In still another case, he found only three cells in the egg-apparatus and seven antipodals. Chiarugi (1925), working on *Tuberaria guttata*, found an abnormal embryo-sac with two synergids, each of them containing three big nuclei and one of them a satellite nucleus also in addition. The egg cell showed the normal form, but the secondary nucleus was divided into three nuclei. Modilewski (1925) recorded a few cases of embryo-sacs in *Allium odorum* showing three synergids, one egg cell, two polar nuclei and five or six antipodals. Maheshwari (1941) mentions a few more cases. According to him,

Gerassimova (1933), in *Crepis capillaris*, and Poddubnaja-Arnoldi and Dianowa (1934), in *Taraxacum kokosaghys*, noted occasional occurrence of 2–4 egg cells in addition to the other elements of the embryo-sac. Martinoli (1939) also occasionally found 9-nucleate and sometimes even 10-nucleate embryo-sacs in *Pyrethrum cinerariæfolium*. The author states that the extra nucleus in the 9-nucleate sac is formed due to a division of the egg and that it (extra nucleus) migrates to the centre of the embryo-sac. In the 10-nucleate sacs, he found three nuclei in the micropylar end, two in the centre of the sac (one of them being the additional cell derived from the division of the egg and the other being the nucleus formed by the fusion of the two normal polar
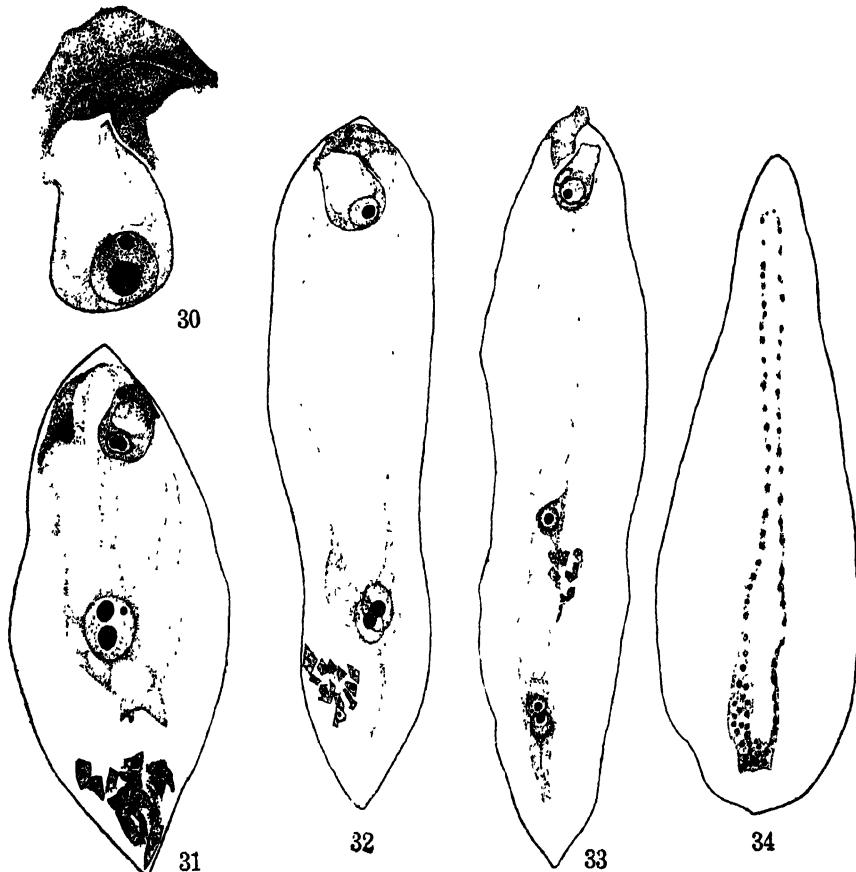
Figs. 30–34. *Thymelæa arvensis.*—Fig. 30. Egg fertilisation. Fig. 31. L.S. of embryo-sac in which the egg fertilisation is completed and the triple fusion is not yet completed. Fig. 32. Embryo-sac showing initial stages of endosperm formation. Fig. 33. Slightly advanced stage, the embryo-sac elongates very much, and the antipodals are left behind. Fig. 34. Nuclear endosperm. Fig. 30, ×1250; Fig. 31. ×560; Fig. 32, ×395; Fig. 33, ×265; Fig. 34, ×53.

nuclei) and five in the chalazal end. P. C. Joshi (1935) found in *Thyla-cospermum rupifragum* in the ovules of an abnormal ovary some abnormal embryo-sacs with 15, 16, 18 and 21 nuclei, in addition to 4- and 8-nucleate sacs. Usually in this species, an 8-nucleate embryo-sac is developed according to the normal type.

OBTURATOR

Early during the development of the ovule, the cells lining the basal part of the stylar tissue grow out into elongated cells and traverse the short space between the micropyle of the ovule and roof of the carpel (Figs. 15 and 16). All these elongated cells are rich in protoplasmic contents and form a more or less compact bundle converging towards the apex of the nucellus. The bundle descends down the funnel-shaped micropyle till it touches the nucellar apex. The presence of similarly developed obturators in other members of Thymelæaceæ has been known for a long time. They differ in their form, but not in origin. For instance, the obturator is more or less compact in *Thymelæa*, *Wikstræmia* and *Daphne*, while it is loosely arranged in *Peddia, Gnidia, Passerina*, etc. An obturator is developed also in the Elægnaceæ but it takes its origin from the funicle. An obturator is also present in Euphorbiaceæ, Rosaceæ, Umbelliferæ, etc., which are unrelated to each other. In all cases, however, it is undoubtedly concerned in directing the pollen tube to the micropyle.

FERTILISATION

The mature pollen grains, when shed, are 3-nucleate. They germinate on the stigma and the pollen tubes run down through the style and obturator and reach the nucellus of the ovule. After entering the embryo-sac, the pollen tube seems to proceed towards one of the synergids and not directly to the egg cell. Double fertilisation and triple fusion occur. One sperm enters the egg cell and is seen to be within the egg nucleus for some time before actually fusing with it (Fig. 30). The fusion between the egg nucleus and one of the sperms precedes the triple fusion (Fig. 31). The two polar nuclei lie side by side for a long time and fuse just before triple fusion.

ENDOSPERM

The endosperm is formed according to the nuclear type. After triple fusion, the endosperm primordium seems to divide near the antipodal end. The first division though not seen in my preparations, seems to be completed soon after the triple fusion and a few endosperm nuclei are always formed much before the division of the fertilised egg (Figs. 32 and 33). The nuclei migrate upwards and the cytoplasm, though scanty, accumulates in the micropylar and chalazal ends but remains thin on the sides. The chalazal accumulation is more prominent and the endosperm nuclei lie embedded in it (Fig. 34). The endosperm nuclei undergo further divisions and increase in number. The endosperm remains cœnocytic for a pretty long time, but ultimately becomes cellular (Fig. 18). The central vacuole, however is never filled up with cells.
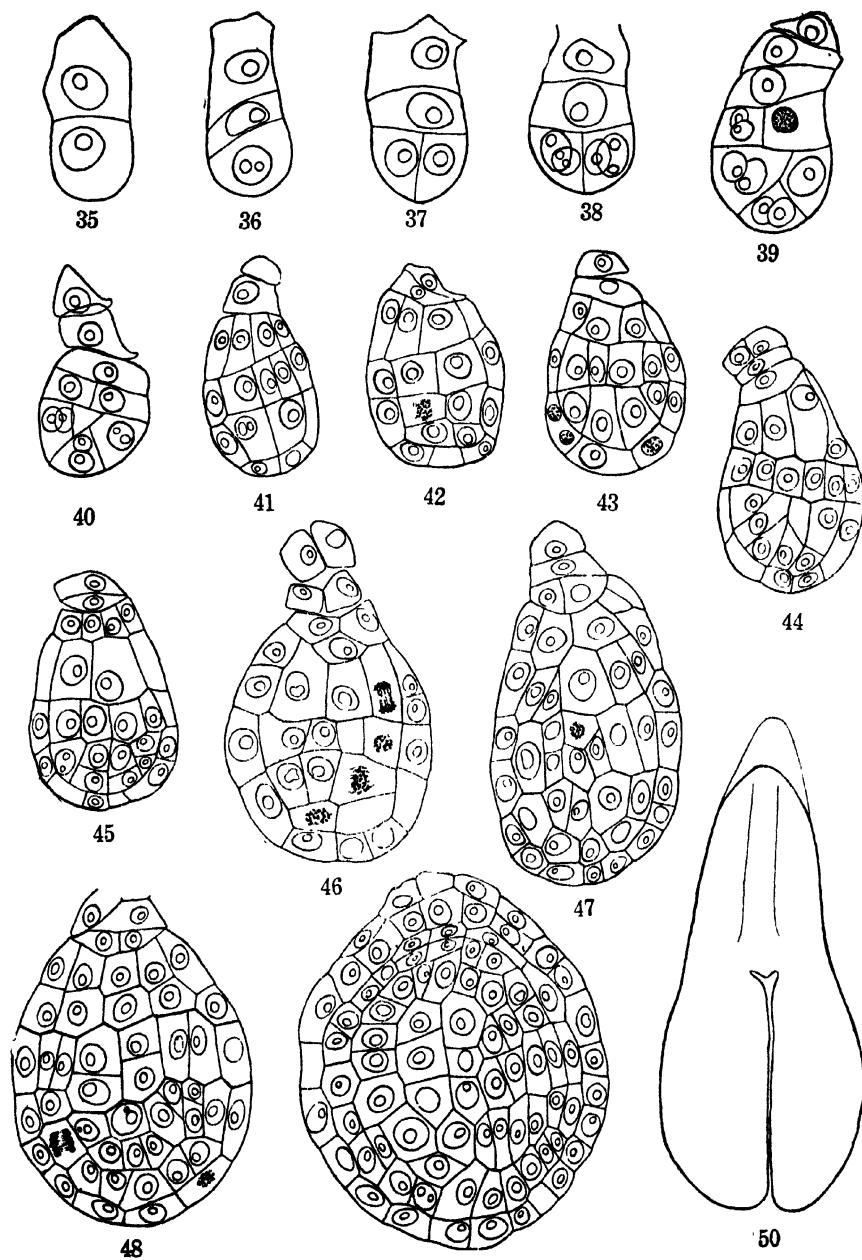
Figs. 35–50. *Thymelæa arvensis.*—Various stages in the development of the embryo. Figs. 35–49, ×560 ; Fig. 50, ×53.

EMBRYO

The first division in the fertilized egg is transverse and takes place only after some endosperm nuclei have been formed. As a result of this division in the oospore, an apical cell and a basal cell are formed (Fig. 35). One more transverse division results in a 3-celled proembryo. No mitotic figure has been observed to enable me to say definitely whether it is the basal or the apical cell that undergoes the second division, but from thickness of the walls separating the three cells, it appears to have taken place in the apical cell (Fig. 36). Fuchs (1938) is also of the same opinion though no actual mitotic figure has been observed even in her preparations. Usually the proembryo remains 3-celled till the appearance of the first longitudinal wall dividing the apical cell (Fig. 37). Further increase in the length of the embryo takes place, always after the appearance of a longitudinal wall in the apical cell as a result of transverse division in either the basal or the middle cell of the proembryo. Due to lack of mitotic figures in the preparations, it is not possible to say definitely whether such further increase in the length of the embryo is due to transverse divisions in the basal or the middle cell of the proembryo.

The embryo proper develops usually from four apical cells of the proembryo (Figs. 40–49). All these four cells, however, are not formed when the first longitudinal division takes place in the apical cell. The III and IV cells from the apex taking part in the formation of the embryo proper are formed at about the time when the sub-apical cell divides longitudinally or soon after it completes the division (Fig. 39). Such a belated differentiation of some cells taking part in the formation of the embryo-proper has been recorded by Kajale in *Boerhaavia diffusa* and *B. repanda* (Kajale, 1938). It is worthy of note that in the above referred species of Nyctagineæ also, four cells of the proembryo take part in the formation of the embryo proper (including hypophysis). The other families of Myrtales, in which detailed development of the embryo is known, differ from Thymelæceæ in the fact that mainly the apical cell takes part in the formation of the embryo proper, the sub-apical cell only contributing to the formation of the hypophysis.

The further development of the embryo also resembles that described by Kajale (1938) in *Boerhaavia diffusa* and *B. repanda*. The four cells taking part form the different parts of the embryo as follows :—

The apical cell forms the cotyledons and the plumule. The second and third cells from the apex give rise to hypocotyl and the major part of the radicle (Figs. 46–49). The fourth cell forms the hypophysis which forms the apex of the radicle (Figs. 44, 46–49). A case however, showing that five cells may also take part in forming the embryo has been noted (Fig. 45). In such a case II, III and IV cell tiers from the apex take part in the development of the hypocotyle and radicle.

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The four cells or the tiers to which they give rise do not develop simultaneously during the differentiation of the embryo. The apical-most is the first one to divide (Figs. 37, 38). Only when it forms the quadrants, the sub-apical one undergoes the first division in a longitudinal manner. It is soon followed by a longitudinal division in the third cell from the apex. By this time the fourth cell may not be even differentiated. The fourth cell is differentiated only when the dermatogen is formed in the third tier (Figs. 42, 44). Their further differentiation is given below :—

The apical cell undergoes the longitudinal division while the proembryo is only 3-celled (Fig. 37). Then one more longitudinal division in it in a plane at right angles to the first leads to the formation of the quadrants (Fig. 38). Till now, the other cells of the proembryo do not show either a transverse division or a longitudinal division. One of the quadrant cells in the apical tier undergoes an anticlinal division (Fig. 39) and the cells get arranged as if separated by oblique walls (Fig. 39). In some of the quadrants of the same tier periclinal divisions begin to take place resulting in the differentiation of the dermatogen (Fig. 40). At about this time the first longitudinal wall appears in the sub-apical cell (Fig. 40) and is soon followed by a longitudinal division in the third cell from the apex. Mostly before the first longitudinal division takes place in the third cell, the differentiation of the fourth cell taking part in the formation of the
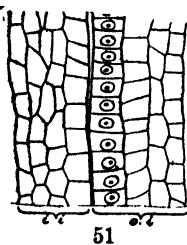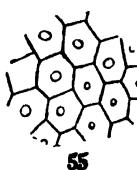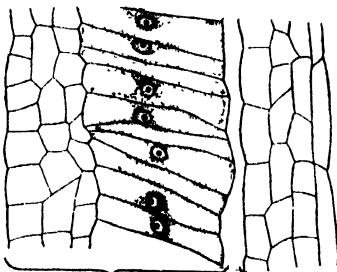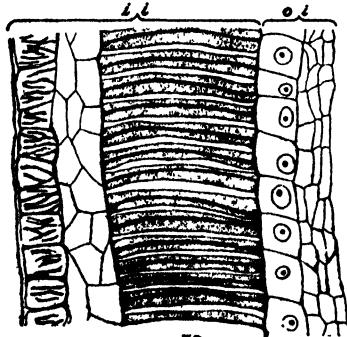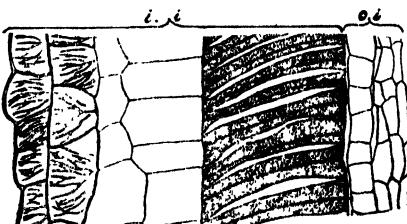
Figs. 51–55. *Thymelæa arvensis.*—Figs. 52–54. Longitudinal sections showing various stages in the development of the seed-coat. Fig. 55. T.S. of woody palisade layer of the inner seed-coat. Figs. 51 and 52, ×373 ; Figs. 53 and 54, ×263 ; Fig. 55, ×373.

embryo proper takes place. The differentiation of dermatogen in the apical cell is soon followed by differentiation of dermatogen in the second and third tiers (Figs. 41, 43). Further multiplication of the cells inside the dermatogen takes place first of all in the apical tier in which first longitudinal or oblique walls are formed (Fig. 44). The cells so formed divide by transverse walls and result in two tiers of cells (Figs. 44–45). Similar multiplication of cells follows in the second and third tiers (Figs. 46 and 47). The cells of the second tier are shorter when compared to those composing first and third tiers (Figs. 44–46).

The differentiation of periblem and plerome follows that of dermatogen. The periblem and plerome are differentiated after a few periclinal divisions in the inner cells, while the dermatogen cells divide only in an anticlinal manner.

The cell lying next to the third cell (or tier) from the apex seems to divide transversely (Fig. 43). The daughter cell towards the apex forms the hypophysis and that towards the base adds to the length of the suspensor. The hypophysis divides anticlinally and periclinally and forms the apex of the root and the root-cap (Figs. 46–49).

The suspensor is very short and is usually made up of 2 or 3 cells (Figs. 44, 46). One, two or all of the three cells, in the later stages, may divide longitudinally and make the suspensor partly or wholly 2-seriate (Figs. 44 and 46). At about the time of the completion of the root-apex or slightly before that time, the cells of the suspensor become loose from each other (Fig. 46). Usually the suspensor cannot be seen in the advanced stages of the embryo. The embryo then grows in size. The cotyledons and stem apex are differentiated at the apex. The embryo is straight (Fig. 50).

STRUCTURE OF THE SEED-COAT

During its development, the embryo destroys the whole of the endosperm and later on the nucellus, but one or two layers of nucellus persist. So there is present a vestige of perisperm in the seed. As already mentioned, due to their growth in the micropylar region, the integuments come together and the funnel-shaped micropyle becomes narrow.

To start with the integuments are mostly three cells in thickness. In the fertilised ovules, each of them becomes four cells in thickness (Fig. 51). The inner epidermis of the outer integument consists of prominent cells which have rich protoplasmic contents (Fig. 51). In later stages of development the cells of the outer epidermis of the inner integument become very much elongated (Fig. 52). In the seed, cells of this layer become very much thickened and their cavities are greatly reduced (Figs. 53, 54 and 55). The cell walls also become lignified and the cells form a woody palisade layer (Figs. 53 and 54). The cells composing one or two of the innermost layers of the inner seed-coat develop fibrous thickenings on their walls such as are seen in the cells of endothecium in the anther wall. The cells composing the outer

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seed-coat become greatly stretched and form the thin outer seed-coat (Fig. 54).

SUMMARY

The structure and development of the anther, pollen, ovule, embryo-sac, endosperm, embryo and seed-coat of *Thymelæa arvensis* Lamk. are described.

The anther development follows the normal course. The wall of the anther at first consists of the epidermis, endothecium, a single middle layer and the tapetum. The epidermis is greatly thinned out due to stretching in mature anthers and the single middle layer between the tapetum and the endothecium gets crushed. The primary sporogenous cells usually become the pollen mother cells. The pollen grains at the shedding stage are 3-nucleate. The sperms are elongated. Starch is present in the pollen grains. The exine shows reticulate sculpture and many germ pores. Two cases of pollen grains with more than three nuclei are described.

The solitary ovule is anatropus and two-integumented. The micropyle is formed by the inner integument. The epidermal cells of the nucellus undergo periclinal divisions and form a 2–3 cells thick cap. The archesporium in the ovule consists of a solitary sub-epidermal cell, which cuts off a primary parietal cell. The latter forms two-layers of parietal tissue which are crushed later by the developing embryo-sac. A linear tetrad of megaspores is formed. The chalazal-most megaspore gives rise to an 8-nucleate embryo-sac according to the normal type. The antipodals increase in number to about 25–30 and persist, although in a degenerate state, a long while after fertilisation. An exceptional embryo-sac with more than 8 nuclei is described.

There is a chalazal conducting strand of elongated cells in the nucellus. An obturator is developed from the base of the style. This descends down into the micropyle and helps to lead the pollen tube towards the embryo-sac.

Fertilisation is porogamous. Double fertilisation and triple fusion occur.

Endosperm is formed according to the nuclear type, but becomes cellular in the later stages. It is completely consumed by the embryo in the mature seed.

Embryo development has been followed in detail. A 3-celled proembryo is formed at first. Four cells take part in the formation of the embryo (including hypophysis), as in *Boerhaavia*. All these four cells, however, are not differentiated at the same time. Only after the apical cell of the embryo completes the first longitudinal division and forms the quadrants the third and fourth cells taking part in the formation of the embryo are differentiated. The apical cell forms the cotyledons and the plumule. The sub-apical cell and the one below it form the hypocotyl and large part of the radicle, while the fourth cell forms the apex of the root and the root-cap.

The seed-coats are two in number. The outer is 4 cells in thickness and membranous. The inner is usually 5 cells thick. Its outermost layer consists of thick-walled palisade cells. The middle cell layers are parenchymatous. The cells of the two innermost layers develop fibrous thickenings.

In conclusion, I wish to experss my sincere thanks to Prof. A. C. Joshi, D.SC., F.N.I., for his helpful suggestions and criticism during the investigation. I am also indebted to him for the material used in this investigation.

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PHYSIOLOGICAL STUDIES ON SOME MEMBERS OF THE FAMILY SAPROLEGNIACEÆ

III. Nitrogen Requirements*

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INTRODUCTION

THE essentiality of a proper source of nitrogen, which is an important limiting factor in the nutrition of fungi, has been well recognised. Experimental data on their nitrogen needs have continued to accumulate during the last 15 years. Robbins (1937) has recently pointed out that fungi fall into four groups, when classified on the basis of their ability to assimilate various forms of nitrogen. According to him "Nitrogen fixing organisms" are such as are capable of assimilating nitrogen as gaseous nitrogen, nitrates, ammonium salts and organic nitrogenous compounds. Under the second group come the "Nitrate ammonium organisms" which are capable of assimilating nitrogen as nitrates, ammonium salts and organic nitrogenous compounds but are incapable of assimilating gaseous nitrogen. The third group consists of "Ammonium organisms" which can assimilate nitrogen as ammonium salts and organic nitrogen but are incapable of assimilating gaseous nitrogen and nitrates. The last category comprises the "organic nitrogen organisms" which are capable of assimilating organic nitrogenous compounds only.

The study of various genera belonging to the family Saprolegniaceæ seems to have been much neglected as regards this important factor. Volkonsky (1933, 1934) studied the suitability of some nitrogenous substances on a few of them but he too did not give a relative value of various nitrogenous substances used by him. A little later Leonian and Lilly (1938) found that *Saprolegnia parasitica* along with some others was unable to grow with ammonium nitrate and required an amino acid for its growth.

The present investigation was undertaken with the aim of clarifying and adding to our knowledge the nitrogen needs of some members of the family Saprolegniaceæ, *viz.*, *Achlya* sp., *Brevilegnia gracilis* v. Eek., *Isoachlya anisospora* var. *indica* Sak. et Bhar., *Saprolegnia delica* Coker and *S. monoica* Pringsh. hitherto uninvestigated.

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METHODS

The methods and technique employed in this investigation were the same as described in an earlier paper (Bhargava, 1945). The basal medium consisted of 0·5 gm. each of KH_2PO_4, $MgCl_2 \cdot 6 H_2O$, 0·17 gm. of Na_2S, 5 gm. of dextrose and 1,000 c.c. of double distilled water. The source of sulphur in the case of *Brevilegnia gracilis* was in the form of K_2SO_4 (0·5 gm. per lit.) instead of Na_2S. Magnesium chloride was prepared by the action of pure hydrochloric acid on magnesium ribbon, the commercial reagent grade chemical (Proanalysis of Merck) being of no value in these experiments, as it contained traces of ammonia.

Because of the prohibitive prices of various amino acids only qualitative experiments were carried out in the first instance. The cultures were grown in culture tubes containing 10 c.c. of the nutrient medium. To compare the relative value of nitrogenous compounds as source of nitrogen only a few representatives were taken for quantitative experiments.

Various nitrogenous compounds (inorganic and organic) were added singly to the basal medium in amounts calculated to furnish 700 mg. of nitrogen per litre. Trihydroxy triethylamine, tyrosin and cystin were tried in 0·1%, 0·1% and 0·05% concentrations respectively because of their low solubility. Peptone was used in 0·1% concentration because of its unknown constitution.

EXPERIMENTAL

To the basal medium were added the following compounds singly before autoclaving :—

Inorganic nitrogen.—

> Ammonium nitrate, ammonium chloride, ammonium sulphate, sodium nitrate and sodium nitrite.

Organic nitrogen.—

> Amino acids :
> > (a) Mono-amino derivatives of aliphatic mono-carboxylic acids—
> > > Glycin, d-alanin, d-valin and l-leucin.
> > (b) Diamino derivatives of aliphatic mono-carboxylic acids—
> > > d-arginin and d-lysin.
> > (c) Mono-amino derivatives of aliphatic dicarboxylic acids— ·
> > > l-asparatic acid, d-glutamic acid and asparagin.
> > (d) Aromatic amino acids—
> > > l-phenyl-alanin and tyrosin.
> > (e) Heterocyclic amino acids—
> > > Histidin, tryptophane and prolin.
> > (f) Thioamino acids—
> > > Cystin and cystein hydrochloride.

Amides : Acetamide.
Amines : Urea and trihydroxytriethylamine.
Proteins : Peptone.

The various media thus obtained were inoculated with the fungi. The basal medium alone served as control. It was found that all the nitrogenous substances, except sodium nitrate, sodium nitrite, glycin, arginin, urea and trihydroxytriethylamine were able to supply nitrogen necessary for the growth of *Achlya* sp., *Isoachlya anisospora* var. *indica* and *Saprolegnia monoica*. In the case of *Brevilegnia gracilis* sodium nitrate, glycin and trihydroxyethyl-amine also supported growth, while acetamide was valueless.

To compare the relative value of some of the easily available nitrogenous compounds, the media were poured in flasks and inoculated with the fungi. Table I gives a résumé of this experiment.

TABLE I

Dry weight (in mg.) of fungal colonies grown on 25 c.c. of the basal medium containing organic and inorganic nitrogenous compounds

Period of incubation = 21 days.   Temperature = 25° C.

Compounds		*Achlya* sp.	*B. gracilis*	*I. anisospora* var. *indica*	*S. delica*	*S. monoica*
Ammonium nitrate	..	12·0	20·0	10·0	5·0	25·0
Amm. chloride	..	10·2	10·6	9·5	3·5	21·6
Amm. sulphate	..	9·8	10·0	8·6	4·5	15·0
Sodium nitrate	..	..	18·3	..	..	..
Acetamide	..	5·0	..	4·3	5·0	3·0
Glycin	..	..	27·3	..	..	..
Alanin	..	12·3	32·6	21·6	3·5	6·5
Glutamic acid	..	15·0	23·0	37·3	15·0	29·0
Asparagin	..	5·8	28·3	13·3	15·0	10·0
Basal medium (control)	..	..	..	..	..	..

The results summarised in Table I show that of all the nitrogenous substances tested, glutamic acid is generally the best and acetamide the poorest source of nitrogen.

Effect of sodium acetate on the utilisation of glycin.—Enhancement of growth of *Leptomitus lacteus*, a watermold, by the addition of glycin in a medium suggested to Schade (1940) that it might prove an available nitrogen source if any suitable carbon could be supplied. He found that an acetate, which is an oxidisable substrate, provides a very favourable source for glycin utilisation in the case of *L. lacteus*. To see if acetate would induce growth of the organisms in this case too (where a good source was already present), the following media were prepared :

1. KH$_2$PO$_4$ ..   .. 0·5 gm.
   MgCl$_2$·6 H$_2$O   ..   ..   .. 0·5 „
   Na$_2$S   ..   .. 0·15 „
   Glycin   ..   .. 3·0 „
   Distilled water   ..   ..   .. 1,000 c.c.
2. Medium 1+glucose   ..   .. 5·0 gm.
3. Medium 2+sodium acetate   .. 2·0 „
4. Medium 1+sodium acetate   .. 2·0 „

These were inoculated with *Achlya* sp., *Isoachlya anisospora* var. *indica*, *Saprolegnia monoica* and *S. delica.* On examining the cultures after seven days of inoculation, it was found that there was no growth.

Effect of molybdenum on the utilisation of nitrates.—Steinberg (1937) in his extensive and well-balanced studies stressed the importance of trace elements in the nutrition of fungi. He found that molybdenum was required in a greater degree by the organisms when a nitrate and not ammonia or organic nitrogen was the source. The following media were prepared to see the possible effect, if any, of molybdenum on the utilisation of nitrate :

1.
KH_2PO_4	..	..	.. 0·5 gm.
$MgCl_2 \cdot 6 H_2O$	..	..	.. 0·5 ,,
Na_2S	..	..	.. 0·15 ,,
Dextrose	..	..	.. 5·0 ,,
$NaNO_3$	..	..	.. 4·2 ,,
Distilled water	..	..	. 1,000 c.c.

Achlya sp., *Isoachlya anisospora* var. *indica*, *Saprolegnia delica* and *S. monoica* which showed no growth on a medium containing $NaNO_3$ were grown on media 1 and 2. It was found that there was no growth in any case.

DISCUSSION

Since the fungi used in the present study are unable to grow on a synthetic medium lacking in the source of nitrogen, it is evident that nitrogen is essential for the growth of these organisms. As to the form of nitrogen suitable for their growth, *Achlya* sp., *Isoachlya anisospora* var. *indica*, *Saprolegnia delica* and *S. monoica* are unable to utilise nitrite or nitrate, but show good response to ammonium as well as organic nitrogen. Therefore, they very well fit in the "Ammonium organisms" placed separately by Robbins (1937). Steinberg (1937) has shown that molybdenum is essential for the activation of nitrate reductase in the reduction processes whereby nitrates are reduced to ammonia. That the non-utilisation of nitrates is not due to the absence of molybdenum is shown clearly by the absence of growth of these organisms on the medium to which molybdenum had been added in the form of sodium molybdate. *Brevilegnia gracilis* behaves differently and is able to utilise nitrates in addition to ammonium and organic nitrogen. It comes under "Nitrate ammonium organisms". This difference may easily be explained on the basis of the different habitat of the fungi ; *B. gracilis* is a parasite on the roots while the other forms are watermolds.

The general opinion about the availability of nitrite nitrogen is that it is toxic for the growth even in dilute solutions, and that if the fungi use it, they do so with difficulty (Ohtsuki, 1936 ; Sakaguchi and Wang, 1936 ; Wang, 1937 and others). Of the 25 fungi used by them, Leonian and Lilly (1938) obtained fair amount of growth of only one organism, *viz.*, *Blakeslee trispora* with sodium nitrate as source of nitrogen.

Of the organic nitrogen, proteins which are the result of a combination of different amino acids are necessary for the building up of the body of an organism. This holds true for fungi as well. Since the organisms studied above are able to utilise nitrogen from NH_4NO_3 or a single amino acid as the only source of nitrogen in a nutrient medium, it can safely be concluded that they manufacture their own amino acids from these substances. Leonian and Lilly (1938) have reported that *Blakeslee trispora, Phycomyces nitens, Pythium oligandrum* and *P. polymastum*, etc., require only one favourable amino acid as the source of nitrogen for good growth. These organisms resemble *Pythium arrhenomanes, P. deliense, P. graminicolum* and *P. mamillatum* (Saksena, 1940) which are able to grow with NH_4NO_3 or one amino acid.

Amines are generally poor source of nitrogen. Schade (1940) also found them valueless for *Leptomitus lacteus*. Volkonsky (1933, 1934) reported that acetamide was not assimilated by *Saprolegnia dioica* while glycocoll and urea were utilised by this fungus.

The best growth on glutamic acid can be attributed, as already explained by Waksman and Lomanitz (1925), to the much larger ratio of the carbon to the nitrogen and that glutamic acid is very favourable to respiration, resulting in the formation of very little volatile acids. This is further explained by the fact that when the ratio between carbon and nitrogen is high, the amount of ammonia produced will be less because the fungus will continue to grow and derive its energy from the available carbohydrate, and ammonia which is a waste product in carbon metabolism will be utilised resulting in greater amount of growth.

That the failure of *Achlya* sp., *Isoachlya anisospora* var. *indica Saprolegnia delica* and *S. monoica* to grow on glycin is not due to the absence of proper carbohydrate or any oxidisable substance is clear from the experiment where these fungi failed to grow on a medium to which acetate had been added in the presence of glycin.

SUMMARY

Achlya sp., *Isoachlya anisospora* var. *indica, Saprolegnia delica* and *S. monoica* are unable to utilise nitrite or nitrates as source of nitrogen but thrive on ammonium and organic nitrogenous compounds. Glutamic acid serves as the best source, while acetamide is the poorest. *Brevilegnia gracilis* is able to obtain nitrogen from nitrates as well. Addition of molybdenum does not help in the assimilation of nitrates. Glycin, which is a good source of nitrogen for *B. gracilis*, is valueless for the growth of others, and remains so even in the presence of an oxidisable substrate.

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STUDIES IN THE BIOLOGY OF WOOD-ROTTING FUNGI OF BENGAL

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I. Introduction

THE rôle of fungi as agents for the destruction of timber was established by Robert Hartig.[20, 21] Wood-rotting fungi are very common in the forests of India, Europe, America and elsewhere. In many cases, a large percentage of timber is found infected. This has led to an increasingly larger number of workers devoting themselves to the study of tree and timber diseases. Among these may be mentioned Ward,[39] von Schrenk,[37, 38] Lyman,[25] White,[40] Rhoads,[31] Long and Harsch,[24] Buller,[15, 16] Fritz,[19] Baxter[4] and Mounce.[26]

The study of cultural characters for distinguishing wood-rotting fungi is also of great importance. Until recently, identification of the causative organism producing the rot was made from the sporophore found on the external surface. They are, however, not always present on rotten wood, and even when present, may not belong to the fungus actually destroying the internal tissues. In such cases, a pure culture of the fungus from the infected wood can be made.

The most important contribution to the study of this subject in India comes from Bose.[6-13] He has given a systematic account of the Bengal Polyporaceæ and described the life-histories and cultural characters of some of them. But a vast number of wood-rotting fungi still await investigation. The present paper makes a contribution to our knowledge of the life-histories and cultural characters of six wood-rotting polypores of Bengal, namely, *Polyporus brumalis* (Pers.) Fr., *P. friabilis* Bose, *P. rubidus* Berk., *P. ochroleucus* Berk., *Polystictus steinheilianus* Berk. and Lév., and *Merulius similis* B. et Br. Geographical distribution and occurrence of these species have been compiled from the reports of Berkeley,[5] Bresadola[14], Butler and Bisby,[17] Lloyd,[22,23] undkur,[27] Oudemans,[28] Petch,[29] Rabenhorst,[30] Saccardo,[33] Theissen[36] and Bose.[6-10]

II. Cultural Methods and Conditions of Growth

A. *Types of culture*

The initial cultures of all the species under investigation were made either from spores or from the tissue of the sporophore. A spore-culture was, however, preferred to a tissue-culture because in the former case the life-history of the fungus from spore to spore could be studied.

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The spore-discharge was obtained from a fresh sporophore on 3% sterile agar contained in the lower lid of a sterile petri-dish, while a trimmed rectangular block of the sporophore was fixed eccentrically to the inner side of its upper lid with the hymenial surface directed downwards and the whole was placed inside a moist chamber for a few hours. The spores, thus deposited, were then transferred aseptically to culture tubes containing *potato-dextrose agar* and within a day or two, several polysporous mycelia were obtained. In *Merulius similis,* polysporous mycelia could not be obtained by this method and monosporous cultures, both by the 'dilution method' and by the 'streak method' were made.

In making tissue-cultures, the technique adopted by Duggar[18] was mainly followed. Fresh, dried sporophores, however, were used, since it was noticed that contaminations, especially bacterial, were large when fresh but water-soaked fruit-bodies were taken. Tissue-cultures were also successfully made from comparatively dry older specimens, but in such cases, a large inoculum was preferred to a small one owing to the likelihood of the former containing a larger percentage of viable mycelia.

B. *Media used*

For the comparative study of cultural characters of these fungi, the following media were used : (1) *potato-dextrose agar* prepared by Fritz's method,[19] (2) *malt-extract agar* (25 gm. agar and 30 gm. malt extract in 1,000 c.c. distilled water), (3) *oat-meal-agar* (25 gm. agar and 50 gm. quaker oats in 1,000 c.c. distilled water), and (4) *Brown's potato-starch agar* (0·2 gm. aspargin, 2·0 gm. glucose, 0·75 gm. magnesium sulphate, 10·0 gm. potato-starch, 1·25 gm. potassium phosphate and 25 gm. agar in 1,000 c.c. distilled water). Eight c.c. of the medium were poured in each culture tube which was then plugged, sterilised and slanted to the same degree in order to provide surface of uniform area. The pH values of the media after sterilisation, were determined by *k*-potentiometric method and found to be 5·2, 5·2, 4·5 and 5·2 respectively.

A new medium for the cultivation of wood-rotting fungi was described by Badcock.[1] According to the author, many wood-rotting fungi which fail to fructify in other commonly used media readily produce fruit-bodies in this medium. This medium was tried successfully but 'pine wood ash' was used instead of 'Scots fir ash' which was not available.

Wood-block cultures were also made both in Roux-tubes and in Erlenmeyer flasks. Normal and healthy pieces of wood of *Mangifera indica, Bambusa arundinacea, Cocos nucifera, Shorea robusta, Ficus religiosa* and *Ceriops Roxburghiana* were cut into convenient sizes ($3'' \times \frac{3}{4}'' \times \frac{3}{4}''$), and cultures were made by Bose's method.[12] Though the fungi under consideration were mainly collected from bamboo stumps, blocks of the wood of other monocotyledonous and dicotyledonous plants were used in order to find out whether the fungi could attack these hosts as well.

C. *Light, Temperature and Humidity*

(1) *Light.*—All the cultures were exposed either to the diffused light of the laboratory or to complete darkness. In the former case, cultures were placed in an inclined position on the shelves of an almirah situated at a distance of about 15 feet away from the window and these received diffused light from morning till evening. For complete darkness, the doors of an almirah were covered with thick black papers in such a way that no light entered into it when the doors were closed.

(2) *Temperature.*—The cultures were subjected to three ranges of temperature, namely, constant temperatures of 22° C. and 33° C. and a variable temperature of 23°–27° C. of the laboratory during the months of November and December, 1941. All wood-block cultures were, however, kept only in diffused light and in ordinary room temperatures of the laboratory.

(3) *Humidity.*—The relative humidity at the above temperature conditions, was determined as 69–75% at 22° C., 35–42% at 33° C., and 35–58% at 23°–27° C.

D. *Number of tubes inoculated*

Culture tubes $6'' \times \frac{1}{2}''$ in size made of neutral glass were used for the study. In order to make the comparative study thorough and at the same time avoiding any risk, three tubes were used for each treatment and the results and conclusions were based on the average of these.

E. *Important diagnostic characters in culture*

The following characters were studied in the cultures of the various species : (1) macroscopic characters including rate of growth, texture and colour of the aerial mycelium, staining and decolouration of the medium, appearance and drying up of glistening drops of liquid, etc.; (2) microscopic characters such as types of hyphæ, their septation, branching, colour, clamp-connection, spore-formation and crystalline material. Descriptions of basidia, basidiospores or other anatomical characters in the fruit-bodies in culture have also been given. All observations were made from fresh mounts in water. Preparations of lactophenol, 50% glycerine, staining with lactophenol cotton blue were also made.

III. DESCRIPTIONS OF SPECIES INVESTIGATED

1. Polyporus brumalis *(Pers.) Fr.*

Geographical distribution

The species is widely distributed over the tropical and temperate regions of the Old World and only in the temperate regions of the New. In North America, it is common in Canada (Manitoba), Lake Superior (Isle Royale), New Hampshire, Michigan, South-West Virginia, Ohio, Iowa, New York, Wisconsin and in the Middle and Upper Carolina. In Europe, it occurs in Great Britain, Spain, France,

Netherlands, Denmark, Czechoslovakia and Esthonia. The fungus is also found in the mountainous forests of Basehberg near Somerset East and Cape of Good Hope in South Africa ; in India, it is found in Bombay, Punjab, Bengal and Orissa. It is also found in Central Asia and Siberia in the North. In Australia, it occurs in New South Wales, Victoria and Queensland.

Occurrence

It has been recorded on several hosts, viz., *Corylus betulus* L., *C. Avellana* L., *Alnus glutinosa* Gaertn., *Betula verrucosa* Ehrh., *Fagus sylvatica* L., *Acer platanoides* L., *Aesculus hippocastanum* L., *Fraxinus excelsior* L., *Tilia cordata* Mill., and *Saccharum munja* Roxb. It has been collected by the authors from suburbs of Calcutta (Behala) where it was growing saprophytically on a dead and fallen branch of a tree.

Fungus in culture   .

(i) *Habit of growth.*—On *potato-dextrose-agar*, young cultures developed a felty mat in most cases and a sub-felty to felty mat in others. The advancing hyphæ formed a colourless appressed subfelty to felty zone. Irregular condensation of the mycelium in young cultures in darkness at 33° C. made the surface uneven. At 22° C. the cultures exhibited faint zonation. Colourless glistening drops of liquid appeared invariably in cultures 7-days-old. Later on, the mat became compact and uniform. Vigorous growth in darkness at 33° C. was manifested by the evident rolling of the mycelium on the bare glass surface. In most cultures, prominent ridges appeared on the mat making the surface uneven. After 10 days of inoculation, the mat in room temperature became grànular at places, though granules in darkness were less than those under diffused light. Later on, however, these granules disappeared. Within a fortnight, the glistening drops of liquid disappeared, growth declined and the mat became thin and appressed. In some cases the mat was covered by a fresh growth of thin, colourless mycelium in old culutres. On *oat-meal-agar*, the mat under all conditions presented a subfelty to felty appearance except at 22° C., where the mat was appressed showing zonation. The advancing zones were distinctly appressed and sodden. The other characteristics as well as the later development of the mat were like that described for the previous medium. On *malt-extract-agar*, the texture was more or less similar to that on oat-meal-agar. In darkness at 33° C. and in diffused light at 22° C. the young hyphæ at the advancing zone were ,parallel and combed like silk. On *potato-starch-agar*, the growth was poorest in comparison to other media. The mats in most cases were thin, appressed and sodden. This texture also persisted in old cultures.

(ii) *Colour*.*—On *potato-dextrose-agar*, colouration appeared after a week's growth, these being shades of cinnamon buff, light vinaceous fawn, pale ochraceous salmon, light ochraceous salmon, sorghum

* According to Ridgeway.[22]

brown and light vinaceous cinnamon. In early stages the colour confined mainly to the inoculum, the hyphæ at the advancing zone remaining colourless. Pigmentation was accentuated in diffused light and tinting in darkness appeared later. After 10-days-growth all the earlier shades disappeared and deeper tint of seashell pink, zinc orange, ochraceous orange, hays russet, mars brown, pale vinaceous drab, light buff, warm buff and orange pink appeared. Colour was best developed in room temperature (23°–27° C.) and for 33° C., pigmentation was denser in diffused light than in darkness. In 22° C. varied colouration was much pronounced in dark than in diffused light, in the latter case, pigmentation developing after a fortnight. Thus, in cold room diffused light definitely retards pigmentation, while darkness accentuates it, but in the other two temperature conditions, reverse was the case. After 21-days-growth, most of the earlier shades persisted, but a deeper shade of liver brown and orange rufus appeared. After a month's time, the shades deepened still further to deep chrome, hæmatite red and vandyke brown. On *oat-meal-agar*, the sequence of colour development as well as the range of colour were like those described above. On *malt-extract-agar*, the intensity and the range of colour were much less. The difference as regards the conditions of light and darkness, if at all present, was very slight. On *potato-starch-agar*, colouration was the poorest and developed after 21-days-growth. The tinting was very light with shades of light buff, pale ochraceous buff, sea-shell pink, pale vinaceous drab, and liver brown. The colour of the medium was changed.

(iii) *Rate of growth.*—The slants were covered with moderate rapidity in all the media, but advance in *potato-starch-agar* was slower in comparison to other media. The rate of growth was highest at 33° C., moderate at room-temperature (23°–27° C.) and comparatively less in 22° C. In general, growth in diffused light was more rapid than growth in darkness.

(iv) *Sporophore production.*—Stalks of fruit-bodies appeared in several cultures under different conditions but the first tube to fructify was on *oat-meal-agar* in diffused light at 33° C. Three stalks arose from the base, two of which were suppressed after some time while the third one developed. Later on, similar stalks appeared in other conditions but comparatively larger number fructified at incubator-temperature. The stalks always had a tendency to branch. In no case, however, pileus was formed. Bits of mycelium from 7-days-old cultures on *potato-dextrose-agar* were transferred to wood blocks in Roux-tubes as well as in flasks. Growth started within a few days with its characteristic colour and zonations as noted on agar media. The mat was thin and appressed. No fruit-bodies, however, developed on wood. Cultures were later grown on the medium advocated by Badcock[1] in Erlenmeyer flasks of 1,000 c.c. capacity each. Normal fruit-bodies with true pilei developed in about a month. A pileus, on sectioning, revealed basidia ($10-12\mu \times 5-6\mu$), each with four sterigmata bearing basidiospores ($4\mu \times 4\mu$).

(v) *Mycelium.*—Hyphæ in young and old cultures are of three types, (*a*) thin-walled, hyaline, with dense protoplasmic contents, septate, profusely branched, clamp-connections numerous, 3–4 μ broad ; (*b*) thin-walled, hyaline, with granular protoplasm, septate, sparingly branched, clamp-connections, about 2 μ broad : and (*c*) thick-walled, hyaline, unbranched and 7–8 μ broad. Chlamydo-spores abundant both in young and old cultures, terminal and intercalary, with granular contents, 10–12 $\mu \times$ 10 μ. Abundant crystals of calcium oxalate are present (Text-figs. 1–5).

2. Polyporus friabilis *Bose*

Geographical distribution

Polyporus friabilis is confined to India and is very common in Bengal. Outside Bengal, it occurs in Madras and Orissa.

Occurrence

The fungus usually grows on humus being associated with rotten leaves and grasses. It has been reported to be growing on *Excœcaria agallocha* L. from Madras. The writers collected the material from dead bamboo clumps and palm stems in Calcutta, Howrah and suburbs.

Fungus in culture

(i) *Habit of growth.*—The mycelium spread rather slowly over the agar surface. On the *potato-dextrose-agar*, inoculum, in most cases, was downy or downy to velvety at the beginning and the advancing zone was evident. In 10 days, the mat became compact and felty. Best growth was obtained at 33° C., moderate in room temperature (23°–27° C.) and comparatively poor at 22° C. In the last case, growth appeared as a more or less circular patch with distinct, appressed and sodden advancing zone. In old cultures, growth was vigorous under all conditions. Rolling of the mycelium over the bare glass surface was vigorous at 33° C., less so in room temperature, while no rolling could be noticed in culutres kept at 22° C. After 35 days of inoculation the superficial mat, in all cases, presented a thick matted felt. Colourless glistening drops of liquid appeared in cultures about a month old in diffused light at 33° C. These drops assumed a beautiful pink colouration in about 2-months-old cultures. Later on, the drops diied up. On *oat-meal-agar* the habit of growth was in the main described as above. The advancing zone at room temperature was appressed and like combed hairs. At 33° C., the upper advancing zone was like a pile of velvet. On *malt-extract-agar*, the growth was comparatively poor. In room temperature, the mat was more compact in diffused light than in darkness. The downy texture gave place to cottony mat, and finally to a smooth matted felt. On *potato-starch-agar*, growth appeared to be poorest, while other characteristics were as described for *malt-extract-agar*.

(ii) *Colour.*—On *potato-dextrose-agar*, no colour appeared in 5-days-old cultures except under diffused light at 33° C., where a patch

of deep safrano pink appeared round about the inoculum. In 10 days, colouration appeared in room temperature and at 33° C. but no tinting was noticed at 22° C. This signifies that for this fungus, a low temperature prevents pigmentation, while a higher temperature accentuates it. Again, since colour appeared earlier in diffused light and later in darkness under any condition of temperature, it suggests that light accentuates pigmentation while darkness retards it. Tinting in young cultures was mainly over the inoculum and consisted of shades of light buff, pale flesh colour to flesh colour and pale salmon colour. Other portions of the mat remained colourless, which, later on, developed pigmentation. The earlier shades, later on, deepened to sea-shell pink, salmon buff, pale ochraceous salmon, pale buff, light buff, light ochraceous buff and pale flesh colour. These shades, however, were mixed with white. In 35 days, most of the shades disappeared and deeper tints of salmon orange, light russet, vinaceous, brownish vinaceous, sweet pink and flesh colour appeared. Colour of the medium was considerably changed in old cultures. On *oat-meal-agar* colouration developed late and no colouration was noticed till the 25th day after inoculation except in diffused light at 33° C. where safrano pink, developed on the 5th day, deepened to light buff on the 10th day. The later shades approached nearly those described for the previous medium. On *malt-extract-agar*, tinting of the mycelium was poor as compared to the previous media, while on *potato-starch-agar*, colour developed only in darkness at room temperature and on diffused light at 33° C.

(iii) *Rate of growth.*—The rate of growth was best on *oat-meal-agar* and on *potato-dextrose-agar*, moderate on *malt-extraxt-agar* and least on *pottato-starch-agar*. The advance was rapid in cultures kept at 33° C., moderately high in room temperature, while at 22° C. the advance was very slow. The dual effect of *potato-starch-agar* medium and low temperature had a marked effect on growth, for no sign of growth could be seen in 25-days-old cultures when growth could be evident only in darkness. Growth in darkness was more than growth under diffused light under all conditions.

(iv) *Sporophore production.*—Bits of cultures from 7-days-old cultures on *oat-meal-agar* were transferred to wood-blocks in Roux-tubes and in flasks. Growth started within a fortnight. In Roux-tubes, growth was very vigorous, giving a thick woolly appearance, some mycelium at the lower part penetrated into water and formed a floating mycelial mat. In flasks, the same woolly appearance was seen. The colouration of the mycelia was typically as seen on agar media. Fruit-bodies were developed neither on agar media nor on wood-blocks.

(v) *Mycelium.*—The hyphæ from culture consists mainly of two types, *viz.*, (*a*) thin-walled, branched, septate, 4–4·7 μ broad and (*b*) thick-walled, branched, septate, somewhat beaded in appearance and 4·7–6 μ broad. Crystals of various shapes are present (Text-figs. 6–7).
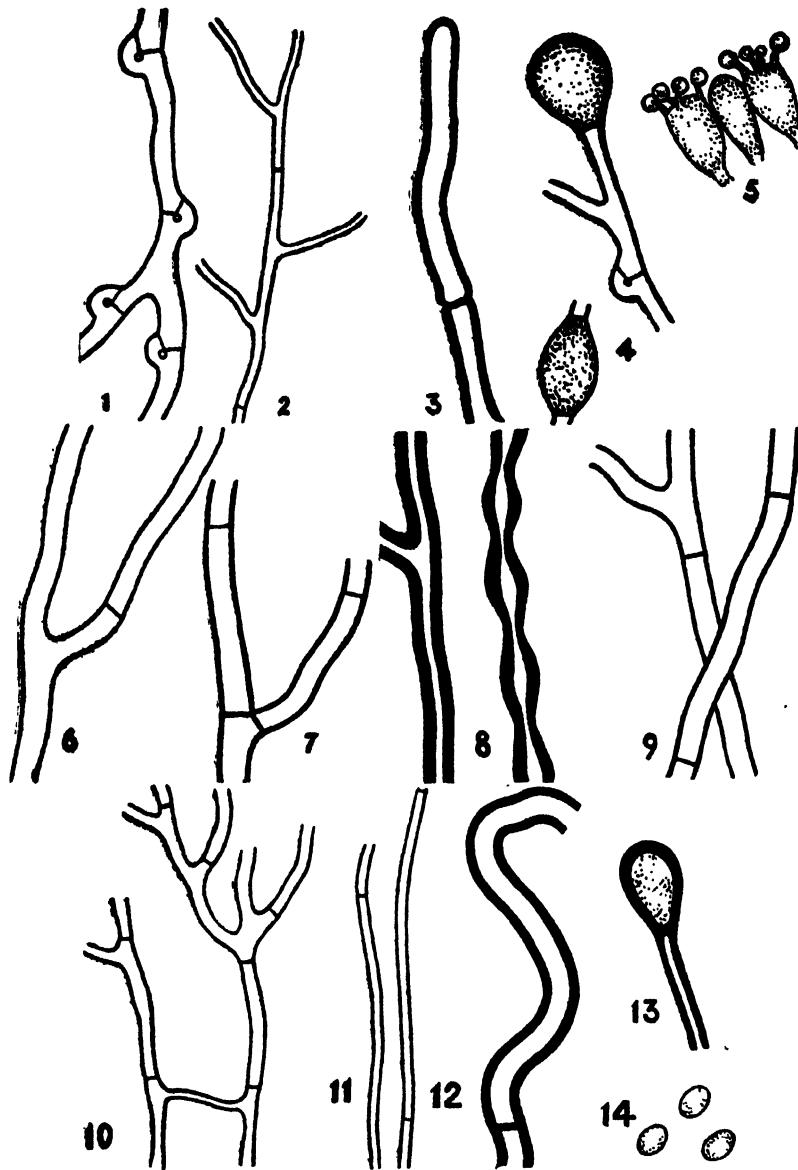
Text-figs. 1–14—Figs. 1–5. *Polyporus brumalis* : 1. thin-walled hypha with clamp-connections; 2. thin-walled hypha without clamp-connections; 3. thick-walled hypha ; 4. chlamydospores ; 5. basidia with basidiospores.   Figs. 6–7.   *P. friabilis* : 6. thin-walled hypha ; 7. thick-walled hypha.   Figs. 8–9.   *P. rubidus* : 8. thick-walled hypha ; 9. thin-walled hyphæ.   Figs. 10–14.   *P. ochroleucus* : 10. thin-walled, much branched hyphæ ; 11. thin-walled, unbranched hyphæ ; 12. thick-walled, unbranched hypha ; 13. terminal chlamydospore ; 14. basidiospores.   (×520).

3. Polyporus rubidus (*Pers.*) *Fr.*

Geographical distribution

The fungus has been collected from Brazil and Rio Grande do Sul in S. America. In India, it occurs in Calcutta and suburbs, Sinchul hills (Bengal) and Lokra hills (Assam). It is also found in Burma, Malaya Peninsula, Java, Lang, Alor and Philippine Islands.

Occurrence

It has been reported to grow on dead wood of *Alnus nepalensis* D. Don from Darjeeling. The authors have collected the fungus from dead bamboo clumps in Sonarpur, 24-Parganas, Bengal.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose-agar*, and on *oat-meal-agar*, the initial growth of the superficial mycelium produced a sub-felty to felty mat with a narrow and appressed advancing zone. Rolling of the mycelium on the bare glass surface in the form of long, weak hyphæ giving a silky appearance began very early in young cultures and is characteristic for the fungus. This rolling was, however, very vigorous at 33° C. Within 10 days after inoculation in room temperature (23°–27° C.) and at 33° C., the mat became compact and felty throughout due to early condensation of the mat with a pile of velvet at the upper advancing zone. At 22° C., there was less condensation of the superficial mat so that it remained loosely felty. The upper advancing zone, moreover, did not exhibit a pile of velvet as stated above. In 35-days-old cultures; the growth characters remained constant under all conditions, each tube exhibiting a semi-lunar raised velvety region at the upper advancing zone. No glistening drops of liquid were observed during the course of study. As the cultures dried up, the long rolling hyphæ began to recede from the base of the glass tube in the form of dry membranous sheet. On *malt-extract-agar*, the texture of the mat was floccose or floccose subfelty to felty texture in about 10 days after inoculation. At 22° C., condensation was less, so that the mat remained floccose. In 25-days-old cultures, the mat became felty in all cases. The other characteristics, namely, the formation of the pile of velvet at the upper advancing zone, rolling, &c., were like those described above.

(ii) *Colour.*—No colour developed under any condition in 5-days-old cultures. On *potato-dextrose-agar*, colour developed on the 10th day after inoculation, the shades being mainly capucine buff, and pale ochraceous buff towards the inoculum and light buff at the upper advancing zone. No colour developed in darkness at 22° C. Later on, the shades deepened to cinnamon buff, sayal brown, and ochraceous tawny. The colour of the medium was changed to mummy brown. The glass surface was stained due to tinting of the rolling mycelium over the bare glass surface. The tinting on the glass consisted mainly of shades of amber brown, yellow ochre, old gold, pinkish buff. Tinting on the pile of velvet at the upper advancing zone consisted of pale ochraceous buff to light ochraceous buff. On *oat-meal-agar* and on

malt-extract-agar, the intensity of colour was much less than those described above. On *potato-starch-agar*, colour production seemed to be least pronounced. A tinge of sayal brown developed at 33° C. in 25 days. Later on, a shade of tawny developed at 22° C. under diffused light.

(iii) *Rate of growth.*—The rate of growth of the superficial mycelium was best at 33° C., moderate at room temperature and comparatively less at 22° C. The slants were covered within a week in room temperature and at 33° C. and within 10 days at 22° C. Growth in darkness, in general, was more vigorous than that under diffused light.

(iv) *Sporophore production.*—Several wood-blocks were inoculated with the fungus both in Roux-tubes and in flasks as usual. Growth started after about a fortnight and attacked the wood vigorously. After about a month, the mycelium condensed at places forming a buff coloured area. No fructification appeared on agar media or wood-blocks.

(v) *Mycelium.*—Two main types of hyphæ can be seen in cultures, *viz.*, (*a*) thin-walled, much branched, contents hyaline, $2 \cdot 5$–$3\,\mu$ broad and (*b*) thick-walled, distantly branched, contents hyaline, contents granular and 3–$3 \cdot 7\,\mu$ broad (Text-figs. 8-9).

4. Polyporus ochroleucus *Berk.*

Geographical distribution

The fungus is widely distributed, being found in the Bahamas and Brazil in S. America ; Portugal in Europe ; Portuguese Guinea and Eastern Cape Forest conservancy in Africa. In India, it occurs in Bengal, Assam (Lokra hills) and after passing through Malaya Peninsula and Molucca Islands, it extends upto Queensland, New South Wales and Tasmania in Australia. It is also found in Ceylon and Japan.

Occurrence

It has been reported to grow on dead logs at the base of the trunk of *Casuarina equisetifolia* L. and on dead trunks of *Lagerstrœmia flos-reginœ* Retz.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose-agar*, the young cultures developed a downy sub-felty to felty texture over the inoculum with broadly appressed and sodden texture over the greater part of the slant surface. The advancing zone was distinct and broadly appressed in all cases. In darkness at 22° C., the cultures exhibited faint zonations round the inoculum. On the 10th day after inoculation the mat remained thin, appressed and sodden with a felty inoculum. In 25-days-old cultures, the mat became a thin and powdery felt, this powdery appearance being less pronounced at room temperature. On *oat-meal-agar*, the texture of the superficial mycelium was like that described above, but the mat was more thick. At 22° C., the mat was

more loose. Colourless glistening drops of liquid appeared in old cultures in diffused light at 33° C. On *malt-extract-agar* and on *potato-starch-agar*, the same mat was presented but in the latter medium, growth was very poor.

(ii) *Colour.*—A tint of warm buff on *malt-extract-agar* and patches of chamois and cinnamon buff on *potato-starch-agar* only developed in 35-days-old cultures. No colour developed on other media, the cultures remaining white throughout.

(iii) *Rate of growth.*—The rate of growth was rather slow in comparison to other fungi. On *oat-meal-agar*, the rate of growth was rapid than on other media. Growth was best at 33° C., moderate at room temperature and comparatively less at 22° C. In general, growth was more rapid in darkness than in diffused light.

(iv) *Sporophore production.*—The wood-blocks were inoculated with 10-days-old mycelium on *potato-dextrose-agar.* The growth on the external surface of the blocks was poor and the mat was thin and slightly yellowish in colour. Tiny fruit-bodies developed in several cultures on agar media. A fruit-body, on section, showed basidia and basidiospores. The spores were oval and measured about $7 \cdot 8 \times 13 \mu$.

(v) *Mycelium.*—Three main types of hyphæ can be distinguished in culture, *viz.*, (*a*) broad, thin-walled, hyaline, branched, closely septate, about $2 \cdot 7 \mu$ broad : (*b*) narrow, thin-walled, hyaline, sparingly branched, septate, about $1 \cdot 3 \mu$ broad and (*c*) thick-walled, sparingly branched, distantly septate, more or less coiled and $2 \cdot 7 - 4 \mu$ broad. Chlamydospores, both terminal and intercalary, are present. The chlamydospores have thick walls showing striations, contain granular protoplasm and measure about $8 \mu \times 5 \cdot 4 \mu$ (Text-figs. 10–14).

5. Polystictus steinheilianus *Berk. and Lév.*

The fungus was identified by Bresadola and at first regarded as synonymous with *Trametes rigida* Mont. and Berk. but later considered as a good species. He also regarded it as synonymous with *Polystictus connexus* Lév.

Geographical distribution

The fungus occurs in Martinique, Brazil and Venezuela in S. America. It is also found in Bengal and Orissa in India.

Occurrence

It grows on dead logs of *Shorea robusta* Gaertn., *Tectona grandis* L. and dead bamboo stem.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose-agar*, the initial growth of the superficial mycelium produced a sub-felty to felty mat. The advancing zone at room temperature was narrow, appressed and sodden. No evident advancing zones could, however, be evident in other conditions. Faint zonations were noticed early in young cultures kept at 22° C. Growth seemed to be the best at 33° C.,

moderate at room temperature and comparatively less at 22° C. Vigorous growth at 33° C. was manifested by the rolling of the mycelium on the bare glass surface in 5-days-old cultures. In 10 days after inoculation, the mat invariably became felty with an appressed to sub-felty advancing zone. At 33° C., the upper advancing zone looked like a pile of felt and numerous glistening drops of liquid appeared towards the base of the tube. Rolling of the mat was evident in all the cultures. In old cultures, the felty mat gave place to a woolly growth, and a pile of felt at the upper advancing zone was prominent. On other media the sequence of texture as the cultures developed was like that described for *potato-dextrose-agar*.

(ii) *Colour.*—On *potato-dextrose-agar*, a tint of light buff to shades of isabella colour appeared in 5-days-old cultures under diffused light at 22° C. No colour developed in other conditions. After 10 days of inoculation, colouration appeared in diffused light at room temperature and in darkness at 22° C., and consisted of ochraceous tawny to buckthorn brown. The shades in diffused light at 22° C. depend to pinkish buff, cinnamon buff, tawny olive, Saccardo's umber and drab. The bare glass surface was stained due to tinting of the rolling mycelium. Thus, for this fungus pigmentation was best developed at 22° C. and least at room temperature. At 33° C. the colour appeared very late. Moreover in diffused light, pigmentation developed earlier than in darkness and also consisted of deeper shades. In 25 days, no new shades appeared, but the lighter shades were absent, while the deeper shades only persisted. Pigmentation was noticed at 33° C. In still old cultures, deeper shades consisting of light clay colour, deep sayal brown, tawny olive, etc., only persisted. On *oat-meal-agar*, the same shades of colour as described above were seen but colouration developed late. On *malt-extract-agar*, the shades were deeper than those seen for *potato-dextrose-agar*. The colour over pore-mouths consisted of shades of light cinnamon drab and light drab. These shades, later on, deepened to hair brown, pinkish buff, gull grey and deep gull grey. On *potato-starch-agar*, the intensity and variety of colours were best as compared to other media. Within 5 days after inoculation, pigmentation developed under all conditions and consisted of various shades of ochraceous tawny, cinnamon brown, olive buff, antimony yellow, yellow ochre, buckthorn brown and warm buff. Thus, the shades were much deeper as compared to other media. On the 10th day, the lighter shades deepened to dresden brown, tawny olive, clay colour, saccardo's umber, pinkish buff, cinnamon buff, sayal brown and mikado brown. Colour over pore-tubes consisted of shades of gull grey to deep gull grey. Intensity of colour seemed to be best developed under diffused light at 33° C. and at room temperature. At 22° C. comparatively less pigmentation was noticed, a fact quite contrary to that seen for the previous media.

(iii) *Rate of growth.*—The initial growth was very rapid and the slants were covered within a week in all cases except for *potato-starch-agar* where the growth was comparatively slow and the slants were covered within 10 days of inoculation. The rate of growth was, however, best at 33° C., moderate at room temperature and at 22° C.

The rate of growth in diffused light was more than in darkness under any conditions of temperature.

(iv) *Sporophore production.*—For the early development of fructification *malt-extract-agar* and *potato-starch-agar* proved to be the best. On *potato-dextrose-agar* and *oat-meal-agar* comparatively less number of tubes fructified. The fruit-bodies were resupinate and were mostly formed at the upper advancing zone. The pore-tubes were irregular and consisted of various shades of light cinnamon drab, light drab, hair brown, pinkish buff, gull grey and deep gull grey. The fruit-bodies were peculiar in that the hymenial surfaces after a time were covered by a fresh mycelial growth developed from the germination of the secondary spores. Bits of mycelium from 7-days-old cultures on *potato-dextrose-agar* were transferred to the wood-blocks of all the plants previously mentioned, in Roux-tubes and in flasks. Growth started within a fortnight and in about a month, most of the wood-blocks were thoroughly infected. The mycelium was seen condensing in patches. The colouration of the mycelium was however very slight. In about 2 months time, fruit-bodies developed in all the wood-blocks both in Rouxtubes as well as in flasks. A fruit-body, in section, showed the presence of hyphal pegs and secondary spores. On adding a drop or two of sterile distilled water aseptically to culture tubes, the same fruit-body showed many immature basidia.

(v) *Mycelium.*—The hyphæ in culture consists of three main types, *viz.*, (*a*) thin-walled, branched, hyaline, septate, with clamp-connections, about $2 \cdot 7 \mu$ broad ; (*b*) thin-walled, rarely branched, hyaline, septate, without clamp-connections, about 2μ broad, and (*c*) thick-walled, branched, without clamp connections and about 3μ broad (Text-figs. 15–20).

6. Merulius similis *B. et Br.*

Geographical distribution

The fungus occurs in Portuguese Guinea, in Africa and Bengal, Assam (Lokra hills), United Provinces, Ceylon and Malaya Peninsula in Asia.

Occurrence

It grows in imbricate clusters on clumps and roots of living and dead bamboos and is very common in Calcutta and suburbs.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose* and on *oat-meal-agar*, the initial growth of the superficial mycelium produced a felty mat with appressed advancing zones. The young cultures exhibited faint zonations at 22° C. as well as in room temperature. Within 10 days after inoculation the cultures became faintly granular due to irregular condensation of the mycelium. Rolling of the mycelium was only evident in darkness at 33° C. The cultures under different conditions of light and darkness exhibited no special distinguishing features. As the cultures became old, the mat became coarsely granular and eventually a compact and smooth felt resulted. Numerous colourless

glistening drops of liquid appeared in darkness at 33° C. and 22° C. These drops, later on, dried up. In darkness at 33° C., there was a pile of felt just behind the upper advancing zone. On the other media the mat remained as a thin felt.
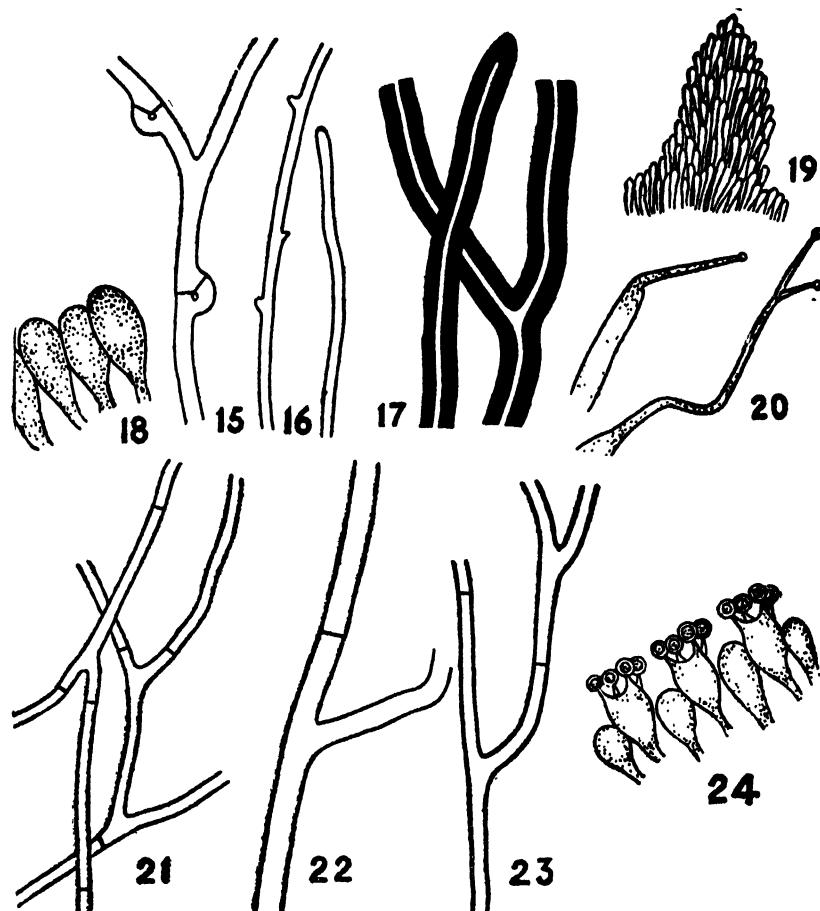
Text-figs. 15–24—Figs. 15–20. *Polystictus steinheilianus:* 15. thin-walled, branched hypha with clamp-connections ; 16. thin-walled, unbranched hyphæ without clamp-connections ; 17. thick-walled hyphæ ; 18. immature basidia ; 19. hyphal peg ; 20. secondary spores. Figs. 21–24. *Merulius similis:* 21. thin-walled, branched narrow hyphæ ; 22. thin-walled, branched broad hypha ; 23. thick-walled hypha ; 24. basidia with basidiospores. (×520).

(ii) *Colour.*—On *potato-dextrose-agar* a shade of light buff developed in the cultures kept at 33° C. in about 10-days-old cultures. This deepened the pale ochraceous buff later on. The mat at the room temperature and at 22° C. remained chalk white throughout. On *oat-meal-agar*, shades of light buff, buff colour and pale ochraceous

buff developed in 10-days-old cultures only under diffused light at 33° C. Later on, these shades deepened to sea-shell pink and maize yellow. On other media, shades of buff, warm buff, mixed with white developed in old cultures at room temperature and at 33° C. under both conditions of light and darkness.

(iii) *Rate of growth.*—The rate of growth was moderately rapid on all the media, being best at 33° C., moderate at room temperature, and a little less at 22° C. Advance was more rapid in darkness than in diffused light.

(iv) *Sporophore production.*—Resupinate patches of fruit-bodies appeared on agar media in about 45 days. The wood-blocks were inoculated with mycelium from 10-days-old cultures on *potato-dextrose-agar*. Within a month the mycelium condensed at places forming a cushion with bright yellow colour. A fruit-body, on sectioning, showed the presence of basidia ($9.75-10.5\mu \times 7.5-8.25\mu$) with four strigmata and basidiospores ($3.75\mu \times 3.75\mu$).

(v) *Mycelium.*—Three main types of hyphæ can be recognised in cultures, *viz.*, (*a*) thin-walled, branched, septate, with granular contents, $1.3-2.7\mu$ broad ; (*b*) thin-walled, branched, septate, with granular contents, $2.7-4\mu$ broad, and (*c*) thick-walled, branched, hyaline and $1.3-2.7\mu$ broad. Calcium-oxalate crystals of various shapes are present in cultures (Text-figs. 21–24).

IV. GENERAL CONSIDERATIONS AND CONCLUSIONS

An attempt has been made to summarise the influence of several external factors which affect the vegetative growth and fruit-body formation in artificial cultures of these fungi. The effect of a single factor has been studied by keeping the others constant.

(1) *Light*

The influence of light on Polypores has been fairly worked out. Long and Harsch[24] allowed direct sunlight to reach the young cultures for one to two hours, but later the amount of direct sunlight was decreased by light screens. This checked the mycelial growth and intensified the colours of the aerial mycelium. Fritz[19] carried on her investigations in complete darkness where the diagnostic characters are accurately manifested. In this investigation the cultures were placed both in diffused light and darkness. Direct sunlight was avoided as its unfavourable action has already been pointed out by Fritz.[19]

The effect of light on the growth and development of each of the fungi has already been described. In the presence of light, the mat becomes more compact due to early condensation and more rich and varied colouration is produced. The writers are of the opinion that although light causes early appearance of pigmentation, it in no way determines the final range of colours, which seems to be constant for each species when grown on a particular medium under definite temperature conditions. Cultures grown in darkness overrun rapidly on the surface of the agar slant.

(2) *Temperature*

Comparative cultural studies were made at ordinary room temperature (23° C.–27° C.) and at constant temperatures of 22° C. and 33° C. This is approximately the same as the range (22° C.–35° C.) used by Fritz.[19] Mounce[26] used a still lower temperature (0° C.–8° C.), which seems to be beyond the usual range.

In general, growth at 22° C. is very unsatisfactory, the mat being thin and loose. Faint zonation is noticed invariably in young cultures of all the fungi. The rate of growth is rather slow. Colour production is delayed and the intensity of pigmentation is much less than that in other temperature conditions. Mycelial growth seems to be best at 33° C., the mat being more compact due to early condensation. The rate of growth is also rapid, pigmentation appears earlier and more intense than in other conditions. At room temperature, the habit and rate of growth are moderate and the range of colour nearly approaches that found at 33° C. These observations agree with those of Fritz[19] and Mounce[26] who also noticed that intensification of colour takes place with increase in temperature. Since during the experiment no intermediate temperature between 27° C. and 33° C. was tried, the writers could not establish the optimum temperature at which growth was at its best but the temperature of 33° C. seemed to be fairly near the optimum.

(3) *Substratum*

(a) *Agar-media.*—Bose[12] and other workers found that *malt-extract-agar* was quite suitable for the display of cultural characters as well as for fruit-body formation. The authors, however, obtained better results on *potato-dextrose-agar* medium. Fritz[19] and Zeller[41,42] also found this medium quite suitable. Fritz[19] found that *potato-dextrose-agar* and *malt-extract-agar* were equally good but on the whole the former was more preferable, since mats were closely interwoven and as a consequence presented surfaces of more definite character. For this reason, she based her *key* by growth on *potato-dextrose-agar*. In diffused light and at a temperature of about 33° C., the medium provides the best condition for growth particularly in the case of Polypores. *Oat-meal-agar* medium proved satisfactory for all the fungi. *Potato-starch-agar*, on the whole, proved to be unsatisfactory except for *Polystictus steinheilianus* in which case colour production as well as development of fruit-bodies were found to be the best. Thus, a single medium has been found which is universally good for all fungi and gives a display of cultural characers of all. Hence, for a comparative study of the cultural characteristics of fungi, it is always advisable to select a number of media, including, at least, one synthetic medium.

(b) *Wood-blocks.*—The wood-blocks were inoculated both in flasks and in Roux-tubes. The advantage of flask culture over Roux-tube culture is that in the former case, the mycelium has a good start for growth and attacks the wood earlier than in the latter. In Roux-tube cultures, the inoculum must include a sufficient amount of medium

so that the mycelium may retain its viability for a long time. It has been observed that the specific nature of the wood is not of much importance and growth takes place on all wood under proper conditions, since *Polystictus steinheilianus* fructified on *Bambusa arundinacea, Shorea robusta, Mangifera indica* and *Ceriops Rox-burghiana.* The fact that no sporophore has ever been found in nature on any of these plants except *Shorea robusta* shows that there must be some factor present in the living wood which has been lost during the processes of drying. Another interesting observation was that *Polystictus steinheilianus* formed fruit-bodies on agar media within a week and thus had a remarkably short life-history. The same fungus, however, took two months to fructify on wood proving that life-histories of hard fungi are prolonged on wood-block cultures, as was pointed out by Bose.[13]

(4) *Effects of various factors on sporophore production*

It is known that the production of fruit-bodies in nature in many Hymenomycetes is more or less dependent upon the influence of light. Spaulding,[35] however, found a few Polypores fructifying in clay mines. Long and Harsch[24] observed that certain members of Polyporaceæ could produce fruit-bodies in complete darkness. In artificial cultures, so far as our observations indicate, sporophores were obtained as frequently in darkness as in light.

Detailed observations on the production of true pilei of *Polyporus brumalis* in artificial culture have been published elsewhere.[3] It was observed that the stalks of the fruit-bodies were positively photo-tropic but had no relation to the influence of gravity. The stalks, however, failed to produce typical pilei on agar media and various treatments of light, temperature and humidity showed that each of these conditions alone had very little effect on pileus-formation. Cultures were later grown on Badcock's medium[1] in Erlenmeyer flasks and it was observed that stalks were formed which reached the bases of the plugs where they remained as such without forming the pilei. As soon as the plugs were opened, the stalks elongated and formed normal pilei. Thus, it appeared that aeration was one of the main factors responsible for the production of normal pilei in *Polyporus brumalis.* In a recent paper Badcock[2] modified his earlier methods and thoroughly discussed the conditions influencing the development of normal fruit-bodies in culture. According to him the following condi-tions appear to be essential : the provision of a generous supply of a rich, well-aerated medium with plenty of moisture ; moderately high relative humidity, but not a saturated atmosphere, at the surface of the medium and around the developing sporophores : and exposure to light of moderate intensity.

Sections through the hymenial surface of the fruit-bodies of *Polyporus brumalis, P. ochroleucus* and *Merulius similis* revealed the presence of basidia and basidiospores. *Polystictus steinheilianus* was remarkable in that the porous surface once formed was later masked by a fresh mycelial growth covering up the entire hymenial surface. This mycelial overgrowth was probably due to germination

of secondary spores which were abundantly found in the sections of such a fruit-body. Bose[13] maintains that a definite percentage of humidity is required for the production of basidia and basidiospores in the fruit-body in artificial culture but with the lowering of humidity, numerous secondary spores are produced. Sections through a part of the fruit-body of *Polystictus steinheilianus* in culture revealed the presence of secondary spores. But when few drops of sterile distilled water were poured aseptically into the culture tube, the same fruit-body, on section, revealed the presence of innumerable immature basidia.

That there is no relation between the appearance of glistening drops of liquid and the formation of fruit-body, was established during the course of the investigation. No doubt in many cases, fruit-bodies appeared immediately after the appearance of liquid drops, but this fact can, on no account, be taken as the cause. In cases of *Polyporus friabilis* and *Polyporus rubidus* glistening drops of liquid appeared and later on dried up, but in no case fruit-bodies developed.

A temperature of about 33° C. in diffused light seemed to be the optimum condition for fruit-body formation. At this temperature, the mat became compact and fructifications developed early.

(5) *Humidity*

To get spore-print from a dried sporophore, the method adopted by Bose[11] was followed. To maintain maximum humidity of the surrounding atmosphere during spore-discharge, moist chamber was made by lining the inside of a bell-jar with moistened blotting paper and placing some water inside the jar. That high humidity provided a favourable condition for vegetative growth was certain but how far the reproduction was retarded or accelerated could not be ascertained. In Roux-tubes, mycelial growth at the lower end of the wood-blocks near the water surface was vigorous and some of them travelled down to the water surface, grew luxuriantly and formed a dense floating mycelial mat. Fruit-bodies when formed on wood in Roux-tubes were seen at the portion of the blocks, away from the water surface.

(6) *Gravity*

The stalks of *Polyporus brumalis* did not respond to the force of gravity. The upward growth of the stalks was purely incidental for in culture tubes kept horizontally it was seen that the vertical stalks arising at the base of the tubes soon turned their apices and grew horizontally parallel to the glass surface.

SUMMARY

1. Six species of Bengal Polyporaceæ, namely, *Polyporus brumalis*, *P. friabilis*, *P. rubidus*, *P. ochroleucus*, *Polystictus steinheilianus* and *Merulius similis* have been studied.

2. The technique of making different types of cultures, preparation of media and the conditions under which the fungi were grown have been described.

3. The descriptions of the fungi including their geographical distribution, occurrence, habit, colour, rate of growth, mycelium in culture, sporophore-production on various media have been given.

4. General observations regarding the effects of light, temperature, substratum, humidity and aeration on vegetative growth and fruit-body formation have been made.

The authors take this opportunity of expressing their deep sense of gratitude to Prof. S. P. Agharkar, Head of the Department, for the facilities given and to Dr. S. R. Bose, Carmichael Medical College, Calcutta, for his valuable suggestions and interest taken in the work.

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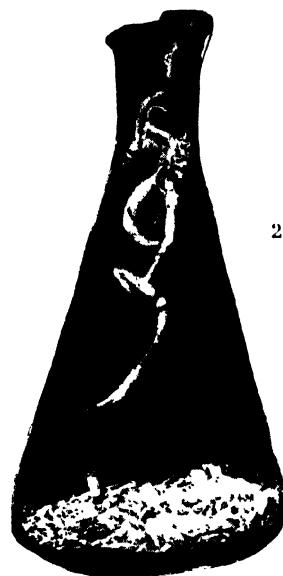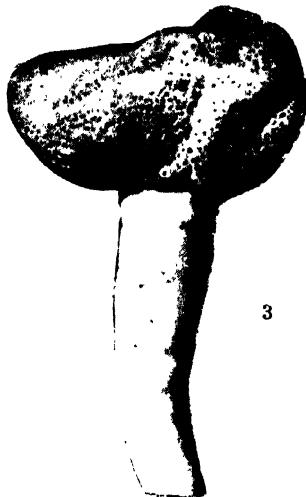
SACHINDRANATH BANERJEE AND BIMAL KUMAR BAKSHI—

STUDIES IN THE BIOLOGY OF WOOD-ROTTING FUNGI OF BENGAL.

EXPLANATION OF PLATES

PLATE II

Fig. 1.   Stalks of fruit-bodies of *Polyporus brumalis* formed on potato-dextrose-agar about 40-days-old (×3).

Fig. 2.   A fruit-body of *P. brumalis* showing typical pileus formed on Badcock's medium (½ Nat. size).

Fig. 3.   A pileus of *P. brumalis* showing hymenial surface with well-developed pores on Badcock's medium (×3).

PLATE III

Fig. 4.   A resupinate fruit-body of *Merulius similis* formed on Potato-dextrose agar about 30-days-old (Nat. size).

Fig. 5.   A resupinate fructification of *Polystictus steinheilianus* formed on malt-extract-agar about 20-days-old (Nat. size).

Fig. 6.   A fruit-body of *P. steinheilianus* formed on potato-dextrose-agar about 15-days-old (½ Nat. size).

Fig. 7.   A fruit-body of *P. stinheilianus* formed on sterilized wood block of *Mangifera indica* about 2-months-old (½ Nat. size).

Fig. 8.   An irregular resupinate fruit-body of *P. steinheilianus* formed on a sterilised wood-block of *Shorea robusta*, about 2-months-old (Nat. size).

SOME FOSSIL LEAVES OF _LITSAEA LANUGINOSA_ NEES. FROM THE KAREWA BEDS AT LIDDARMARG, PIR PANJAL, KASHMIR

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INTRODUCTION

IN the year 1911, Mr. C. S. Middlemiss of the Geological Survey of India during his geological studies in Kashmir, made a collection of about 200 specimens of fossil plants from Liddarmarg, a small village situated on the densely forested northern slopes of the Pir Panjal Range, which rises on the southern side of the valley in a series of high mountain chains.

The fossiliferous beds, which have yielded a fossil flora of 30 genera and 40 species (Puri, 1941, pp. 7-8) belong to the Lower Karewa formations. These deposits on recent geological evidence, are considered to be Lower Pleistocene in age. At first they were considered to form a part of the Upper Karewas (see de Terra and Wodehouse, 1935, p. 5) which are of the Upper Pleistocene Age, but later on de Terra (see de Terra and Paterson, 1939, pl. 55 : pp. 109–114) changed his views in favour of the Lower Pleistocene Age for these beds. This view was long ago hinted at by Middlemiss (1911), when he referred the fossil beds at Liddarmarg as "Older Karewas".

The plant-bearing outcrops occur at an altitude of 10,600 ft. above sea-level and, as Middlemiss (_loc cit._, pp. 121–22) writes, "are exposed in two stream beds near the present Gujar (herdsmen) encampment of Liddarmarg (lat. 38° 48′ ; long. 74° 39′). The dip is of the usual slightly inclined character, and the beds were found to contain a flora of well preserved leaves." This collection was examined by Middlemiss who, with the help of Mr. I. H. Burkill, was able to identify most of the fossil

leaves and announced (*loc. cit.*, p. 122) the occurrence, among the collections, of a few leaves of *Cinnamomum*, one of which according to them was specifically identical with *C. Tamala* Nees., a moderate-sized tree of the tropical and sub-tropical Himalayas.

The entire collection of Middlemiss, together with the original identifications, was kindly lent to me in 1939 by the Director, Geological Survey of India, for study at Lucknow under the guidance of Professor B. Sahni, F.R.S. On my examination of this material I find that a large number of the fossil leaves were unnamed and most of the original identifications of the named specimens the labels of which were fortunately preserved with the fossils, on comparison with my identifications have proved to be erroneous. A comprehensive description of the fossil flora discovered in this material, and another collection made in 1932 by Dr. H. de Terra from a different spot in the same locality, will be published in a series of papers at a later date. But a short note dealing with the description of hitherto unfigured and incorrectly identified leaves supposed to be of "*Cinnamomum Tamala*", but which are identical in all respects with living leaves of *Litsæa lanuginosa* Nees., is given in the following few pages.

DESCRIPTION

Phylum	..	Angiospermæ.
Subphylum '	..	Dicotyledonæ.
Division	..	Archichlamydeæ.
Order	..	Laurales.
Family	..	Lauraceæ.
Genus	..	*Litsæa.* '

Litsæa lanuginosa Nees.

(Pl. IV, Figs. 1–4)

The leaf fragments described below are poorly preserved impressions embedded in a fine-grained, compactly set clays, which are mostly greyish black in colour. The clays are composed of irregularly bedded layers, several inches thick, and it is, therefore, rarely possible to recover a complete leaf by splitting bigger blocks along the plane of bedding.

The entire collection before coming into my hands was heavily coated with some preservatives applied with a view to preserve the specimens as Middlemiss (*loc. cit.*, p. 122) wrote "there by painting them at Mr. Blyth's suggestion, with gelatine first and canada-balsam varnish afterwards, I was able to preserve a large number showing all the delicate venation, serrated edges, and in one or two instances even the deep red tints of the original fallen leaves".

Plate IV, Figs. 1–3 show natural size photographs of three leaf fragments, which represent the basal half of leaves, the apical parts being entirely missing in all. The shape of the fossil leaves cannot be definitely ascertained on account of their fragmentary nature but by comparing them with living leaves of this species it seems probable

that they had oblong-lanceolate lamina. The fragments show a slight variation in size. The lamina measures 2·5″ long by 1·1″ in the broadest part in the smallest specimen (Fig. 1) and it is 2·5″ × 1·4″ in Fig. 2. In the largest fragment (Fig. 3) it is 4·85″ × 1·9″. By a comparison of the fossil fragments with living leaves it is suggested that the broadest part in a fragment is probably the middle part of the lamina from which it gradually narrows down into a wedge-shaped base seen prominently in Fig. 2. The base in Fig. 3 is slightly distorted and curved to one size, due probably to a break in this region of the leaf, which might have been caused before or at the time of its deposition. In this specimen a bit of the petiole is also preserved. The margins are entire ; the apex is broken in all specimens and cannot be definitely ascertained but it seems to have been acute as in living leaves.

The venation is apparently pinnate but a closer examination reveals it to be strongly sub-triplinerved at the base. A strong midrib, which has left a fairly deep groove in Figs. 1, 2 and stands out in the form of a ridge in Fig. 3, runs in the lamina dividing it into slightly unequal halves. A comparison of the fossils with living leaves, in which the midrib is marked out on the lower surface, it is suggested that the photographs 1 and 2 are impressions from the lower surface and Fig. 3 is from the upper surface of the leaf. Two or three pairs of prominent secondaries arise in the upper part of the fragments at acute angles, in an alternate manner, on either side of the midrib. They follow a straight course in the lamina and are ending abruptly in the margins. The basal pair of secondaries, which is about half as thick as the midrib, arises from the midrib at very acute angles a short distance above the base and runs as far as middle of the lamina and finally ends in the margins far higher up from its point of origin. In one specimen (Fig. 2) a small secondary rib arises very close to the base on one side of the midrib and follows a straight course in the lamina. The secondaries in one specimen (Fig. 1) are quite close to one another but they are fairly wide apart in other specimens. The second basal lateral on the left side in Fig. 2 arises at a distance of 1·2″ from the basal pair. Tertiary and finer reticulations being poorly preserved in all specimens are not brought out in any of the photographs.

The fossils were confused and referred to *Cinnamomum Tamala* Nees., by Middlemiss and Burkill who failed to notice the differences in the details of venation between the two. Plate IV, Fig. 5 is a natural size photograph of a living leaf of *C. Tamala* which is given here to show contrast in venation with fossils and living leaf of *L. lanuginosa* (Pl. IV, Fig. 4). In addition to the basal pair of secondaries present in the fossils as well as in living leaf of *C. Tamala*, the former have two to three additional pairs of laterals, which are altogether absent in the latter. Each of the two basal secondaries in *C. Tamala* arises from the midrib at acute angles and runs in the lamina, as far up as the apex, making an arch parallel to the margin the convexity of the arch facing outwards and finally ends near the apex. In the fossils the basal laterals while running up do not make arches but follow a straight course as far as middle of the leaf and finally end in

the margins about half-way from the base. In details of venation, shape, size, margins, etc., our fossil fragments are identical with living leaves of *Litsæa lanuginosa* Nees. (see Fig. 4), a tropical tree of the sub-Himalayan regions.

Number of specimens.—Three.

Occurrence.—Liddarmarg at 10,600 ft., in the Pir Panjal Range, Kashmir.

Collector.—C. S. Middlemiss, 1910.

Registered Nos. of figured specimens.—

    Pl. I, Fig. 1 : G.S.I. No. K 14/951 (II).
    Pl. I, Fig. 2 : G.S.I. No. K 14/948a27.
    Pl. I, Fig. 3 : G.S.I. No. K 14/951 (I).

MODERN DISTRIBUTION OF *Litsæa*

The genus *Litsæa* is at the present time confined mainly to the tropical regions of Asia and also occurs rarely in boreal parts of America. In Asia, it occurs in the Himalayas, Burma and extends in the south-easterly direction through Malaya Peninsula and Malaya Islands, into tropical and subtropical forests of Australia, New Zealand and New Caledonia. It also spreads northwards from the Himalayas and occurs commonly in North as well as South China, extending as far north as Japan. Towards the south it spreads into Ceylon.

MODERN DISTRIBUTION OF THE FOSSIL SPECIES

Litsæa lanuginosa, which belongs to the section *Neolitsea* of the genus, is definitely tropical in its modern distribution and occurs in India in the outer Himalayan ranges fairly scattered from Kashmir to Sikkim. It is extremely rare in the north and north-western outer Himalayas and according to Parker (1918, p. 430), who is considered to be an authority on the forest botany of these mountains, the species is absent from the Sutlej westwards. However, an authentic sheet in the Herbarium of the Forest Research Institute, Dehra Dun, shows that the species was collected from Muzaffarabad in Kashmir at an altitude of 4,000 ft. Eastwards, it occurs sparingly in sheltered places between the altitudes of 2,000 and 4,000 ft. above sea-level. It has been collected at Suni along Sutlej river and is also recorded from Simla District near Kalka. Further eastwards, it ascends to higher altitudes and has been collected from Rispana Valley, Mussoorie, at 5,800 ft. and occurs at 6,000 ft. above sea-level in the Sikkim Himalayas. It has been collected in this region from several places in Garhwal, Naini Tal, and Nepal, growing in shady places or occupying cooler aspects of these hills. The species is recorded from as far east as Khasi Hills and grows at an altitude of 8,000 ft. in Manipur, Assam. So far as we know, it has never been found growing or collected from Kashmir Valley, the northern slopes of the Pir Panjal Range, the southern slopes of the Main Himalayas, Murree Hills or the Kagan Valley, and its absence at the present time from all parts of Kashmir, excepting Muzaffarabad, and neighbouring mountain ranges is striking. Although our information

regarding data on the modern distribution of the species is based on literature and authentic sheets in the Herbaria yet its absence from these regions is not improbable and seems to conform to the prevailing climatic conditions in this part of the Himalayas.

CONCLUSIONS

The present discovery of *Litsæa lanuginosa*, a tropical species of the Lauraceæ, from Liddarmarg at an altitude of 10,600 ft., furnishes further data in favour of the theory of the Himalayan uplift during the Pleistocene. Its occurrence in the fossil beds at such high altitudes, where it has never been found growing at the present time, is a direct proof to show that the plant-bearing beds have been uplifted since the time *Litsæa lanuginosa* lived in the valley, which must have had a tropical climate at that time. In fact the prevalence of a tropical climate in the Kashmir Valley during Lower Pleistocene times has already been conclusively proved on palæobotanical evidence by the author (1943) and the present discovery lends further support to the idea of an extension of tropical and sub-tropical montane forests in the Kashmir Valley, from where they have now disappeared on account of the changed climatic conditions brought about by the uplift of the Pir Panjal Range.

The incorporation of the present species in the Liddarmarg flora in place of *Cinnamomum Tamala* has not materially altered the general features of the flora, which still continues to indicate a tropical climate as before.

SUMMARY

The paper describes some fossil leaves of *Litsæa lanuginosa* Nees., a tropical species of the Lauraceæ (wrongly referred by I. H. Burkill and Middlemiss to *Cinnamomum Tamala* Nees.) from C. S. Middlemiss's collection of 1910 from the Lower Karewa beds (Pleistocene), which are exposed along two streams near Liddarmarg (alt. 10,600 ft., Pir Panjal Range, Kashmir Valley). The fossils were lent to the author for study by the Director, Geological Survey of India.

L. lanuginosa is confined at the present time to the tropical parts of the Outer Himalayas from the Sutlej eastwards between the altitudes of 2–4,000 ft., but it ascends to the altitude of 5,800 ft. in Rispana Valley, Mussoorie. Further east it occurs in Garhwal and Nepal, and ascends to 6,000 ft. in Sikkim. It is recorded from as far east as Assam in the Khasi Hills and has been collected from as high an altitude as 8,000 ft. from Manipur. It is conspicuous by its absence from North and North-Western Himalayas and does not grow anywhere in Kashmir excepting Muzzaffarabad at 4,000 ft. It is also absent from the neighbouring regions including Pir Panjal Range, the Kagan Valley and the Murree Hills.

This sharp contrast in the past and present distribution of the species in Kashmir lends additional support to the theory of the Pleistocene uplift of the Himalayas, already confirmed by the author on palæobotanical evidence. The idea of an extended occurrence of

1a

a tropical and sub-tropical forest flora into the Kashmir Valley across the Pir Panjal Range during the Lower Pleistocene times thus gains further ground.

This incorporation of *Litsœa lanuginosa* in the fossil flora in place of *Cinnamomum Tamala* does not materially change the general feature of the flora.

ACKNOWLEDGMENTS

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EXPLANATION OF PLATE IV

The figures are from untouched negatives and are of natural size.

Litsœa lanuginosa Nees.

Fig. 1. Fossil leaf fragment (impression of the lower surface).

Fig. 2. Fossil leaf fragment (impression of the upper surface).

Fig. 3. Fossil leaf fragment (impression of the lower surface).

Fig. 4. Modern leaf for comparison with the fossils and contrast with modern leaf of *Cinnamomum Tamala* Nees.

Cinnamomum Tamala Nees.

Fig. 5. Modern leaf to show contrast in venation with the fossil fragments and modern leaf of *Litsœa lanuginosa* Nees.

G. S. PURI—

SOME FOSSIL LEAVES OF LITSEA LANUGINOSA NEES. FROM THE
KAREWA BEDS AT LIDDARMARG, PIR PANJAL, KASHMIR

STUDIES IN THE DISEASES OF *MANGIFERA INDICA* LINN.

Part V. On the Die-back Disease of the Mango Tree

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INTRODUCTION

MANGO trees are subject to a disease in which the leaves fall off, twigs dry up, and the entire branch or the part affected assumes an appearance of dry, dead, leafless twigs sticking up from among the green healthy foliage resembling the ' die-back ' and twig blight of trees commonly described in pathological literature. The disease is prevalent not only in the U.P. but also in other mango-growing provinces of India. A number of diseased twigs were collected from Lucknow and Madras, and the investigation was undertaken to ascertain the cause of the disease. The results are presented in this paper. A preliminary note was published earlier (Das Gupta and Zachariah, 1939).

SYMPTOMS

General.—The effect of the disease on the general appearance of the trees is noticeable at anytime of the year ; but it is most conspicuous during the months of September and October. A large number of wilted branches and twigs are prominent among the green foliage of infected trees. In advanced stages of the disease leafless branches as well as twigs showing different stages of wilt give the tree an appearance as if it has been scorched by fire (Pl. V, Fig. 1). A magnified view of a portion of tree affected by the disease is shown in Pl. V, Fig. 2. Generally the smaller twigs and branches of comparatively old trees are affected.

External.—The first external evidence of the disease is the discolouring and darkening of the bark at a certain distance from the tip (Pl. V, Fig. 3*a*). The front and side views of the dark infected area are shown in Pl. V, Figs. 8 and 9. Such dark patches usually occur in the young green twigs and are hardly distinguishable in older branches.

As the darkening advances towards the tip the leaves just above the infected region wither (Pl. V, Fig. 3*b*). The upper leaves lose their healthy green colour and gradually turn brown (Pl. V, Fig. 4*b*). The browning starts at the base of the leaf, spreads along the midrib, and then out along the veins to the margin. This is followed by the browning of the whole leaf accompanied by the upward rolling of the margine (Pl. V, Fig. 5*a*). Eventually the affected twig or branch

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dies and shrivels up (Pl. V, Fig. 4). A magnified view of the rolled shrivelled leaves is shown in Pl. V, Fig. 6. The brown rolled leaves often persist for a month or more (Pl. V, Fig. 7) and finally drop off, leaving the shrivelled twig altogether bare of leaves which is the characteristic of the advanced stage of the disease (Pl. V, Fig. 7*b*). Infection may be accompanied by the extrusion of gum (Pl. V, Fig. 9*j*).

HISTOPATHOLOGY

The infected twig shows an internal discolouration which is found to extend at an earlier stage of the disease about an inch on either side, towards the tip and the base of the twig, beyond the external darkened bark. The diseased twigs when cut out slantingly along the long axis through the infected region reveal a brown streaking of the vascular tissues, namely, cambium and phloem (Pl. V, Fig. 10). The internal discolouration is diffuse and uniform and appears as a dark streak between the stele and the cortex (Pl. V, Fig. 11). Series of sections of affected twigs showing different stages of the disease were examined to study the effect of the disease on the internal tissues.

Sections at about four inches below the growing point of a twig at a very early stage of infection which appeared healthy except for a short discoloured area on the stem showed slightly shrivelled, epidermal and sub-epidermal cells. The internal discolouration was manifested by the browning of certain regions of the cambium and phloem, where some of the cells were found to be plugged with a yellow, gum-like substance. A few hyphæ were seen in the xylem vessels. The inner regions of the cortex appeared unaffected while the cells of the outer layers had started shrivelling.

In very advanced stages of the disease, the cells of the different tissues of the stem were badly shrivelled. The xylem vessels were plugged with fungal mycelium. The stele and the outer layers got separated from each other along the discoloured band at the cambial region where the cells had disintegrated. Numerous hyphæ were found in this region. A few hyphæ were also found in the cells of the cortex. The mycelium was found not only in the bundles of the stem, but also in the petioles and midribs of leaves of infected twigs.

EXPERIMENTAL

Infected twigs were collected from trees of the Botany Department, Lucknow University, and the Isabella Thoburn College Orchard, Lucknow. Material from Madras was collected by Dr. T. S. Sadasivan from trees showing variable symptoms of die-back.

Twigs showing different stages of the disease were chosen for the investigation. In some the leaves were just turning brown ; in others they had completely shrivelled up. In very advanced stages the twigs were quite dry with no leaves on them. The surface of the bark showed leaf-scars, lenticels and breaks caused by natural cracking of the outer layers.

As isolation from infected twigs by taking small inocula from the internal diseased tissue and directly culturing them in nutrient

medium proved impracticable, the diseased twigs were first placed in moist chambers and the hyphæ growing out from the pieces were sub-cultured in the standard synthetic medium.

The infected twigs were first cleaned by swabbing with cotton-wool dipped in a saturated solution of borax. They were, then, cut into small pieces, 1–1½ inches long and each piece was separately washed in a saturated borax solution, steeped in 0·1% mercuric chloride for about five minutes, shaking vigorously at intervals and was finally washed in three changes of sterile distilled water. After this surface sterilisation, the twig pieces were placed in sterile moist chambers. A large number of twig pieces were tried with wood and bark intact, while other pieces had the bark stripped off from the wood portion and placed in separate moist chambers. Within 3–5 days these twig pieces showed a profuse growth of hyphæ in them. On an external examination only two types of hyphæ could be distinguished, one being pure white, the other whitish in colour at the beginning and turning dark with age.

Separate inoculations were made with hyphæ arising from different parts of the twigs pieces namely cut ends, surface of wood, inner and outer surfaces of bark, on standard synthetic medium and malt agar. By this method altogether five fungi were isolated, *viz.*, *Botryodiplodia theobromæ* (B), *Phoma* (P), *Fusarium* (F), *Fusarium* (f), *Pestalozzia* (Pz), *Alternaria* (A).

The results are shown in Table 1.

TABLE 1

Locality	Twig 1			Twig 2			Twig 3			Twig 4			Twig 5			Twig 6		
	b&w	b	w	b&w	b	w	b&w	b	w	b&w	b	w	b&w	b	w	b&w	b	w
Botany Department	B	B	B	BF	BF	B	PF	PF	F	P	fA	P	P	Pz,f	P	PF	fA	F
Isabella Thoburn College	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Madras	B	B	B	B	B	B	B	E	B	B	B	B	B	B	B	B	B	B

b & w = bark and wood,   b = bark,   w = wood.

On examination of the result it was found that all the six twigs belonging to different trees of the Isabella Thoburn College Orchard and those belonging to Madras showed the presence of *Botryodiplodia theobromæ* alone both from bark and wood whereas the twigs from the Botany Department garden gave anomalous results. The twig 1 produced only *Botryodiplodia theobromæ* from all its parts, the twig 2 produced *Botryodiplodia theobromæ* from wood only, but in bark, and bark and wood *Fusarium* was associated with *Botryodiplodia theobromæ*. Twigs 3, 4, 5 and 6 were characterised by the total absence of *Botryodiplodia theobromæ*, instead of which were found four different fungi,

Phoma, Fusarium, Alternaria and *Pestalozzia* either singly or variously associated. The last three were invariably associated with bark. In each case, however, the wood portion produced only one kind of fungus, for example, *Fusarium* in the wood of twigs 3 and 6 and *Phoma* in that of twigs 4 and 5.

It was apparent from this preliminary experiment that so far as the twigs from Isabella Thoburn College and those from Madras were concerned, the disease was due to *Botryodiplodia theobromæ*. But the appearance of more than one fungi from the wood of different diseased twigs of the mango tree of the Botany Department indicated that each of these fungi may be a potential parasite causing the disease.

Detailed work was next undertaken to confirm the results obtained : (*a*) by studying the spatial distribution of the fungal strains in diseased twigs, and (*b*) by inoculation experiments.

Spatial distribution of fungi.—The same experimental method as described before was employed. Twigs showing various stages of the disease and collected from localities already mentioned were surface sterilized. The diseased, and in some cases, apparently healthy parts were then divided into pieces 1–1½ inches in length (whenever possible bark was separated from wood) and put in moist chamber. The spatial distribution of the fungi in the twigs was found out by noting and drawing the exact position of the piece on the twig and numbering the successive pieces from the tip towards the base. A few leaves of each twig, too, were put in moist chamber after surface sterilisation. The fungi appearing from the twig pieces and leaves were inoculated on standard synthetic medium.

Although a very careful record was made of all the fungi which appeared from bark, and wood of different twigs tested, it was obvious that only the fungi growing out of the wood region, would be responsible for the disease. The fungi arising from bark or the surface of the wood would provide with subsidiary evidence regarding the causal organism. While analysing the results, therefore, greater attention has been paid to fungi isolated from wood portion.

Botany Department Garden.—Five twigs were employed, all belonging to the same tree. The description of the twigs indicating the extent to which disease has progressed is given below :

Twig 1 (Text-fig. 1).—The upper part of the twig had been affected while the lower region remained green and apparently healthy. The affected part of the twig was slightly shrivelled and discoloured. There were no leaf-scars or wounds except a few superficial cracks of the bark. The leaves had wilted and turned brown. The twig was divided into five pieces.

Twig 2 (Text-fig. 2).—This twig showed a more advanced stage of the disease. The stem as well as the leaves had completely dried up. The stem showed a few leaf-scars, lenticels and a scar left by a broken branch. It was divided into seven pieces.

Twig 3 (Text-fig. 3).—This twig showed a very advanced stage of disease and the leaves had fallen off from one of the branches. There

were no leaf scars or wounds but lenticels were present. The upper end had a scar left by a fallen branch. It was divided into eight pieces. Four leaves were also tested.

Twig 4 (Text-fig. 13).—The whole twig had been diseased and it showed a very advanced stage. The twig was completely dry and shrivelled. Leaf scars, branch scars and lenticels were found on the stem. All the leaves had fallen off except for a few on two small branches and these leaves, too, were brown and dry. The twig was divided into 24 pieces.

Twig 5 (Text-fig. 5).—The entire twig had dried up and most of the leaves had broken off. Leaf scars and lenticels were present on the stem. The twig was divided into 10 pieces.

It will be seen from Text-figs. (1, 2, 3 and 13) that three out of the four twigs (Figs. 1–3) have produced *Botryodiplodia theobromæ* from wood from almost all the pieces. In twig 1 (Text-fig. 1) *Botryodiplodia theobromæ* has been found in the wood from the tip up to the base, excepting for one piece right at the end that gave only *Fusarium*. The two leaves tested from the top also gave rise to *Botryodiplodia theobromæ* only.

In twig 2 (Text-fig. 2) again *Botryodiplodia theobromæ* was present in the whole length of the diseased wood of both the branches tested, except two pieces at the end where mixed with *Botryodiplodia* there was *Phoma* in one and *Fusarium* in the other. The petiole of one leaf arising from the twig also gave *Botryodiplodia*.

In twig 3 (Text-fig. 3) *Botryodiplodia* was found all along the wood in all the three branches except at the dry shrivelled end of one branch from which all the leaves had fallen off. This piece gave rise to *Fusarium*. All the four leaves tested also gave rise to *Botryodiplodia*.

Twig 4 (Text-fig. 13), however, which was completely shrivelled gave entirely different result. *Phoma* was found in the wood of all the branches right up to the petiole of the leaves, which were still persisting but along with this fungus was found associated in certain places, *Fusarium*. Comparison with bark fungi showed that *Fusarium* in all these cases must have come from bark.

Twig 5 (Text-fig. 5) also showed practically the same result as twig 4. *Phoma* was present almost throughout the diseased twig, excepting *Fusarium* and *Alternaria* in restricted regions.

It is apparent from the result that in the first three twigs it is the *Botryodiplodia theobromæ* that causes the disease, the other associated fungi are secondary. In twigs 4 and 5 *Phoma* seems to be the casual fungus and *Fusarium* only secondary. The two diseases in these two sets of twigs should therefore be considered as different. This disease due to *Phoma* has only been found in dry shrivelled twigs at the last stage of the disease and no early stage of infection by *Phoma* has been found. It is still to be seen if these may represent the blight disease of mango twig described by Kanitkar and Uppal (1939).
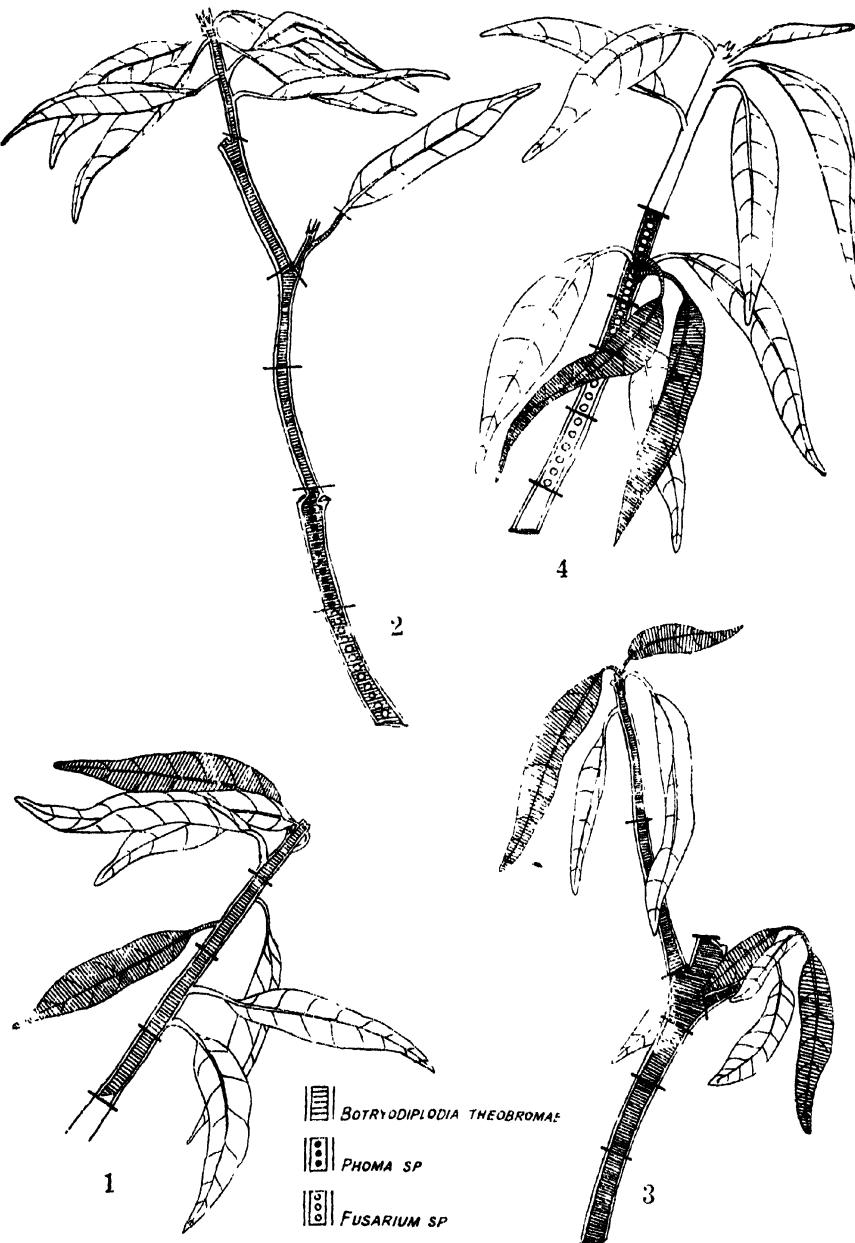
Text-Figs. 1–4. Figures illustrating spatial distribution
of the fungi in the diseased mango twigs
Figs. 1–3. Twigs from Botany Department. Fig. 4. Twig from Isabella
Thoburn College.

Isabella Thoburn College Orchard.—A large number of twigs were collected of which only six twigs from one tree, and two from two others representing various stages of the disease were selected for investigation.

Tree 1, *Twig* 1 (Text-fig. 6).—The twig appeared healthy except for about two inches towards the lower end where it had turned black and slightly shrivelled. In this infected region there were leaf-scars and splits of the bark. The apparently healthy part of the twig was green and had a number of leaves and leaf-scars. The leaves though green were slightly pale and unhealthy looking.

The infected region was divided into two parts, the bark was removed from wood and each was put in a separate moist chamber.

Tree 1, *Twig* 2 (Text-fig. 4).—The only evidence of infection in this twig was slight blackening of the bark at the lower node where two of the leaves had wilted. A small globule of gum was secreted in between the petioles of these two leaves. All the other leaves remained green and apparently healthy. The lower part of the twig was divided into four pieces.

Tree 1, *Twig* 3 (Text-fig. 7).—This twig showed the initial stage of the disease. The infected region had turned black and two leaves at the upper end of the discoloured region had completely wilted. The part of the twig above the infected region had started wilting and the leaves were turning brown. The infected region was divided into 3 pieces and the apparently healthy part towards the apex into 4 pieces.

Tree 1, *Twig* 4 (Text-fig. 9).—A slightly advanced stage of the disease was shown in this twig. The upper end of the twig was discoloured and a brown colour extended along the midrib of the leaves of the infected region. The two edges of the leaves folded inwards and in some cases there was also a twisting of the leaves towards the leaf-scars, lenticels and splits in the bark. Globules of gum were found at different spots. The diseased portion of the twig was divided into six pieces and three of the leaves were also tested.

Tree 1, *Twig* 5 (Text-fig. 10).—The upper end of the twig was infected and the stem had turned brown. Leaf-scars, lenticels and a scar left by a fallen branch were present in the infected region of the twig. Gum was secreted a little below the branch scar. In the leaves, a brown colour extended along the midrib and margins which advanced inwards. The leaves rolled inwards and in some cases they had curled towards the tip. The infected region was divided into 8 pieces and three of the leaves were tested.

Tree 1, *Twig* 6 (Text-fig. 11).—This twig showed a more advanced stage. Leaf-scars and lenticels were present in the infected part and a big globule of gum was secreted towards the lower end of the infected region. The leaves were all brown and shrivelled. The twig was divided into 9 pieces.

Tree 2, *Twig* 1.—A more advanced stage of the disease was shown by this twig. The stem as well as all the leaves had completely dried and shrivelled, and one branch was devoid of leaves. The twig was divided into 18 pieces.

Text-Figs. 5–8. Figures illustrating spatial distribution
of the fungi in the diseased mango twigs

Fig. 5. Twig from Botany Department. Figs. 6 and 7. Twigs from Isabella
Thoburn College. Fig. 8. Twig from Madras.

Tree 3, *Twig* 1 (Text-fig. 12).—This twig showed a very advanced stage of the disease. The entire twig was completely dry and most of the leaves had broken off from the stem. It was divided into 15 pieces.

It will be seen from Text-figs. 4, 6, 7 and 9 to 12 that *Botryodiplodia theobromæ* is the most frequently occurring strain on eight different twigs belonging to three different trees. Twig. 1 (Text-fig. 6) shows the initial stage of infection and the infected region produces only *Botryodiplodia theobromæ.* The result from twig 2 (Text-fig. 4) is somewhat different although the twig is in early stage of infection. Two fungi are found associated together—*Botryodiplodia theobromæ* and *Fusarium. Botryodiplodia* is exclusively present in the leaves, and in the upper region of the infected part, but *Fusarium* extends to the lower portion of the diseased tissue where there is no *Botryodiplodia.*

In twig 3 (Text-fig. 7) *Botryodiplodia* is restricted to the lower region, *i.e.,* the diseased part of the twig. *Fusarium* on the other hand is restricted to the wood of the upper region which externally appeared to be free from disease. The leaves, however, show a mixture of both the fungi.

In twig 4 (Text-fig. 9) where the disease had advanced further the leaves and the entire length of the wood gave *Botryodiplodia,* only a small portion showed the presence of *Fusarium.*

In twig 5 (Text-fig. 10) the wood of the upper portion along with the leaves gave *Botryodiplodia,* lower half only *Fusarium.*

In twig 6 (Text-fig. 11) showing still more advanced stage of the disease, almost the entire length is *Botryodiplodia* which is mixed with *Fusarium* only at the lower end. There is pure *Fusarium* further down.

In twig 1 of tree 2 *Botryodiplodia* is present all along the wood except at the tip and in the small portion at the middle where *Fusarium* and *Phoma* are found.

In twig 1, tree 3 (Text-fig. 12) the entire wood of the infected part showed only *Botryodiplodia theobromæ.*

An analysis of the result shows that there is an overwhelmin evidence that the disease in all these twigs is caused by *Botryodiplodia.*

Madras twigs.—Twigs obtained from Madras were all dry and showed an advanced stage of the disease. Five twigs were tested. All of these gave identical results. Text-fig. 8 shows one of these twigs. It was divided into 10 pieces and the bark was separated from wood in all cases except in the topmost piece. Two of the leaves in two different branches of the twig were also tested. *Botryodiplodia theobromæ* was the only fungus obtained from the diseased bark, wood and leaf (Text-fig. 8) and undoubtedly the cause of disease.

Inoculation experiments

Mango plants.—Forty mango plants were raised from seeds and grown in pots for inoculation tests to find out whether *Botryodiplodia, Fusarium* and *Phoma* could parasitise vigorously growing healthy seedlings. Monohyphal agar cultures of the different strains were used in all the inoculation experiments. The plants were inoculated after

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BOTRYODIPLODIA
THEOBROMAE

PHOMA SP

FUSARIUM SR

they had grown for five months. Most of the plants were about 1½ feet high while a few were only 1 foot in height.

The places to be wounded were first cleaned by swabbing with 0·1% mercuric chloride and then with sterile distilled water. An incision exposing the cells of the wood was made with a sterile scalpel and mycelium taken from the margins of agar cultures 3 days' old in *Botryodiplodia*, and 5 days' old in other 2 strains, was inserted in the wounds. Incised plants which received no inoculum served as controls. The wounded places were protected by wrapping with moist cotton and waxed paper. Of the 40 seedlings used, 10 replicates were made for each fungus—*Botryodiplodia, Phoma* and *Fusarium*, and the remaining 10 served as control.

None of the seedlings showed any sign of infection or wilting even though they were kept under observation for 6 months. A similar experiment with 40 seedlings next year also yielded negative results. Only *Botryodiplodia* was found to have produced in a few twigs slight canker near the inoculation point.

Mango twigs.—Botryodiplodia, Phoma and *Fusarium* were inoculated on healthy young twigs attached to the tree. Twenty-four twigs were selected for the purpose on the same tree. Six twigs were inoculated with each fungus and six served as control. Procedure adopted was the same as in inoculations on the young plants.

Out of six twigs inoculated with *Botryodiplodia*, three showed die-back symptoms on the 12th day after inoculation. All the twigs inoculated with the other strains remained unaffected. The three affected twigs on reisolation yielded only *Botryodiplodia*.

It will be seen from these inoculation experiments that all the four strains have failed to produce the disease on the inoculated young plants while *Botryodiplodia* is the only successful strain producing an infection of 50% on the inoculated twigs. Further inoculation experiments are in progress to find out the predisposing factors and the conditions under which the wounded parts get infected.

<p align="center">·</p>

DISCUSSION

The die-back of fruit and other trees is of common occurrence. Among stone fruit trees the disease is known to occur on almonds, apricots, peaches and plums (Cunningham, 1925), the causal organism, being *Clasterosporium carpophilum*. Die-back of apple branches due to *Glœosporium* sp. has been described by Wilkinson (1942).

The disease is also known to occur on Asiatic chestnuts which is caused by *Phomopsis* (Bedwell, 1937) and *Cryptodiaporthe castanea* (Tul.) Wehmeyer, *Botryosphœria ribis chromogena* G. and D. and *Diplodia* sp. (Fowler, 1938).

The white elm trees (*Ulmus americana* L.) in Nebraska is affected by die-back caused by *Cephalosporium* later identified as *Dothiorella ulmi* (May, 1931). The same disease in Elms in Minnesota is caused by a fungus which appears to be *Cytosporella*.

As regards Gymnosperms, Curtis (1926) reported a die-back of *Pinus muricata* and *Pinus radiata* by the fungus *Botryodiplodia piniea* in New Zealand. The die-back of fir (*Pseudotsuga Douglasii* Carr.) has been attributed to the attack of *Sphæropsis Ellisii* (Petri, 1913) and of *Pseudotsuga laxifolia* to that of *Diplodia pinea* (Waterman and Miller, 1936).

In India Petch (1916) has investigated the die-back of *Hevea brasiliensis* and die-back of tea plant of Ceylon which are ascribed to *Botryodiplodia theobromæ.* Sundararaman (1932) has reported die-back of cashewnuts by *Corticium salmonicolor.* This fungus according to him, is also known to attack mango, orange, jack-fruit, tea, coffee and several other trees. Narasimhan (1933-34) has reported *Diplodia* die-back of limes. Recently Kheswalla (1936) has reported die-back disease of fruit trees in Baluchistan by *Cytospora.* From a die-back of the tops of young Robusta coffee trees Mayne (1936) has isolated *Colletotrichum coffeanum.* Kanitkar and Uppal (1939) have given a short account of the twig blight of mango tree in Poona caused by species of *Phoma.*

In all these cases mentioned the disease is of fungal origin. But die-back may also be caused by bacteria, and by deficiency of salts.

The die-back of *Aucuba japonica* (Thunb.), for example, is caused by *Pseudomonas aucubicola* (Trapp, 1936). Ark and Thomas (1940) described the twig blight of apple tree in California in which he failed to find any pathogenic organism. On the other hand, the addition of boron and potassium in soil was found to reduce the die-back symptoms of the affected plants. Die-back of cloves in Zanzibar Protectorate is stated to be due to phosphorus and potassium deficiency owing to the deflection of these salts by grasses (*Ann. Rep. Dept. Agr. Zanz. Protectorate*, 1935, 1936).

Dwyer (1937) reports die-back deficiency disease in *Cocos nucifera* and also a physiological trouble affecting young palms which is characterised by a pronounced tip withering of the central leaves and drying-back of the outer leaves and pale-brown streaks on the back of the petioles.

Tubbs (1937) investigated factors affecting the die-back disease of tea, ascribed by Petch to *Botryodiplodia theobromæ* and is of opinion that the disease is of physiological origin and is associated with the deficiency in the production of carbohydrates ; tea plants producing only half the carbohydrate necessary for their growth.

A number of fungi has been found to be associated with the die-back of mango trees here investigated. Leaving aside those which are exclusively found on the bark and are definitely saprophytic, there are three fungi *Botryodiplodia, Phoma* and *Fusarium*, which have claim to be regarded as causal organisms. Association of a large number of fungi in a tree affected with die-back is not unknown. Bedwell (1937) found *Sphæropsis, Diplodia, Cytospora, Diplodina, Macrophoma Fusicoccum, Dothiorella, Phoma* and *Epicoccum*, associated with twig blight of Asiatic chestnut, along with the more important pathogen *Phomopsis.*

The reconstruction of the exact position of the fungi in the twig pieces showed that *Botryodiplodia* was exclusively present in the wood of a number of twigs suggesting that the disease is due to *Botryodiplodia*. The pathogenicity of *Botryodiplodia* has also been demonstrated by inoculation experiment in which the fungus was able to infect mango twigs through wounds although no vigorously growing plant showed such infection.

It appears that the disease of other two twigs of the Botany Department is caused by *Phoma*. Although the result of the preliminary inoculation experiment is negative, the fact that the fungus has been found to be permeating the wood of the entire diseased twig almost exclusively seems to indicate the pathogenicity of *Phoma*. Further inoculation experiment is necessary to settle the point.

Kanitkar and Uppal (1939) have also found a species of *Phoma* causing twig-blight of mango trees. It is evident from the spore size that the two species are different.

The die-back or twig blight of the mango trees is caused definitely by *Botryodiplodia* and probably also by *Phoma*. It is not rare to find more than one organism causing die-back of the same species of tree. White elm in different parts of America is a case to the point.

The pathogenicity of *Fusarium* is, however, doutbful. There is no diseased twig from which the fungus alone has been isolated. When associated with other fungi, *Botryodiplodia* or *Phoma* it has almost always been found in restricted regions, the other fungus predominating. In such cases *Fusarium* must be considered as a secondary organism.

The same *Fusarium* found in the twigs of Isabella Thoburn College is pathogenically more significant particularly in two cases.

In tree 1, twig 2 *Fusarium* is not only associated with *Botryodiplodia* at the diseased portion of the twig but also occupies the wood of the diseased portion at the base, where no *Botryodiplodia* has been found. Of more significance is the twig 3 of the same tree. Here *Botryodiplodia* is restricted to the diseased portion only, whereas *Fusarium* exclusively occupies the apparently healthy portion of the twig from above the diseased portion right up to the tip.

The presence of *Fusarium* in advance of *Botryodiplodia* in the apparently healthy tissue up to the tip may point to its being a pathogen, or it may only indicate, that once having penetrated in the wake of the actual pathogen, *Fusarium* can advance more quickly inside the tissue.

There is thus a gradation in the pathogenicity of *Botryodiplodia*, *Phoma* and *Fusarium*, the three fungi intimately associated with die-back. The two fungi *Botryodiplodia theobromæ* and *Phoma* have often been found to be separately associated with *Fusarium*. The possibility that in such cases the disease is due to the combined activity of the two fungi, although *Botryodiplodia* and *Phoma* may produce the disease independently cannot be completely overlooked.

Botryodiplodia has been known to cause die-back in various plants, for example, *Pinus*, rubber, tea, etc., and also associated with leaf-break

of palm leaf, ring disease of palm nuts (Dwyer, 1937), diseased pods of cocoa (Baker, 1936) and storage diseases of grape fruits (Wardlaw and Leonard, 1937). It is not definite, however, if in all these cases the disease is due to *Botryodiplodia*.

The production of the die-back may be occasioned by unfavourable conditions acting as predisposing factors. In such cases the adverse conditions affect the growth and vitality of the tree in question which easily succumb to the invasion of otherwise harmless facultative parasites.

Münch (1935) is inclined to believe that the die-back of Larches is due to a fungus but not the fungus *Dasysclypha Willkomii* commonly associated in Germany with Larch Canker. Grimm (1937), however, is of opinion that the cause of dying off of Larches in Germany was due primarily to the disturbances of transpiration associated with adverse environmental factors ; the trees thus weakened are readily accessible to infection by the Canker producing fungus. Day (1937) supports Langner's general conclusion (1936) that the fungus plays a secondary but a definite and necessary part in the development of the die-back and states that frost in this case is the predisposing factor.

Mayne (1935–1936) found that in the die-back of coffee tree the diseased shoots invariably showed the presence of *Colletotrichum coffeanum*, which was frequently the only fungus isolated. It was always found in shoots showing the very earliest external symptoms of the disease. But from field observations and the inconclusive inoculation experiments and other evidence he came to the conclusion that the primary predisposing factors in die-back are unfavourable conditions and premature leaf fall due to coffee-leaf-disease-fungus *Hemileia vestatrix*, and *Collectotrichum coffeanum* is only secondary to predisposing factors.

Müller (1936) describes that in the top die-back of coffee the presence of abundant shade of *Leucæna glauca* was observed to minimise the incidence of the disease. It is not improbable that high temperature in this case is the predisposing factor.

According to Dade (1937) swollen shoot of Cocoa is the result of extreme exposure of the tree to sun and wind during dry season, brought about by the gradual disappearance of shade trees and the surrounding forest (drought die-back). These weakened trees are invaded by secondary fungi such as *Botryodiplodia theobromæ* and saprophyte such as *Gliocladium roseum*, none of which can attack healthy tissue and produce necrotic die-back tissue with which these are always associated.

In the die-back of mango trees here investigated it seems that the fungi *Botryodiplodia* and *Phoma* are able to infect and produce the disease only in the less vigorous plants or twigs. It can well be that abnormal high summer temperature, 115° C., which sometimes kills twigs outright, is the predisposing factor.

The mode of infection and general symptoms of the disease are almost the same as described for other trees.

The infection in the "top die-back of coffee" caused by *Rhizoctonia* invariably commences in growing twigs and may frequently be detected before the hyphæ spread into stem.   In the field the fungus travels from the leaves, through the wood vessels of the branches and stems. As a rule its diffusion is two to three times more rapid in an upward than in a downward direction (Muller, 1936).

In *Hevea brasiliensis*, the infection usually takes place not at the tip but at a variable distance from the growing point.   The branch dies above the point of attack and less rapidly also backwards towards the base.   As spread backwards occurs, the whorl of branches lower down are killed off in succession.

In the die-back of mango twigs caused by *Botryodiplodia* it has been found that the infection occurs at a node at variable distance below growing point, and the part of the twig above and below the point of infection dies.   The leaves lose their healthy green colour and gradually turn brown.   The browning starts at the base of the leaf and spreads along the midrib and then out along the veins to the margins. This is followed by the browning of the whole leaf, accompanied by the upward rolling of the margin.

In the white elm, the infection twigs show an internal streaking of the vascular tissues of the current season's growth.   Goss and Frink (1934) have failed to find any external blackening of the bark above the point of infection although it has been described by May (1931) to occur on the Dutch Elm.   In the infected mango twigs, the external blackening as well as internal streaking are clearly marked.   The latter is seen as dark band between the xylem and cortex.

SUMMARY

The paper deals with the die-back disease of mango trees.

Botryodiplodia theobromæ, Phoma and *Fusarium* are the three fungi intimately associated with the die-back.

There is direct evidence from inoculation experiments that mango twigs can be infected and die-back produced by *Botryodiplodia.*

The evidence regarding the pathogenicity of *Phoma* is indirect, and based on the almost exclusive appearance of the fungus from some diseased twigs.

The pathogenicity of *Fusarium*, the other fungus associated with the disease, is not established.

These fungi are unable to attack vigorous healthy plants.

The predisposing factor in the case of die-back of Lucknow plants may be high summer temperature which affects the vitality of plants and enables the pathogens to attack.

The external symptoms and histopathology of die-back caused by *Botryodiplodia* have been given in detail.

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EXPLANATION OF PLATE V

Figs. 1–2.   Illustrating general aspect of a mango tree from Madras affected with die-back.

    1.   A diseased tree showing dead shrivelled twigs bare of leaves among green foliage.

    2.   A close-up view of the diseased tree in Fig. 1.

Figs. 3–5.   Illustrating external symptoms of early stage of die-back.

    3.   (*a*) Discolouration and shrivelling of the infected region of stem.   (*b*) Leaves above the point of infection withering.

    4.   (*a*) Shrivelled young twig ; (*b*) upper three leaves have wilted .

    5.   (*a*) Infection at a node where all the leaves have wilted ; (*b*) the leaves at the top apparently healthy.

Figs. 6–7.   Illustrating external symptoms of advanced stage of die-back.

    6.   Characteristically curled leaves on the shrivelled twig enlarged.

    7.   Final stage of dead twigs. (*a*) Bunches of dry, curled leaves at the top ; (*b*) twigs devoid of leaves.

Figs. 8–10.   Illustrating external symptoms at the early stage of infection.

    8.   Stem showing discolouration of the bark at the infected region—dark against green stem. $\times 1 \cdot 5$.

    9.   Side view of Fig. 1 showing the discoloured infected region and the gum globule (*g*). $\times 1 \cdot 5$.

    10.   Twig cut slantingly exposing the wood and internal streaking in phloem (*p*) and cambium (*c*). $\times 1 \cdot 5$.

Figs. 11–12.   Histopathology : advanced stage.

    11.   Section of old infected region. $\times 52$. (*a*) Brown deposits in phloem and cambium ; (*b*) xylem vessels with fungal hyphæ.

    12.   Xylem vessels (of Fig. 11) enlarged to show the hyphæ inside. $\times 330$.

S. N. DAS GUPTA AND A. T. ZACHARIAH (MISS)-

STUDIES IN THE DISEASES OF MANGIFERA INDICA LINN

STUDIES IN THE DISEASES OF *MANGIFERA INDICA* LINN.

V. The Structure and Development of Lenticels in the Mango Fruits

BY S. SINHA

INTRODUCTION

MANGOES have recently received much attention in India and various diseases of the mango fruit are being investigated. The role of fruit lenticels as passages of infecting organisms has been emphasized by several workers (Kidd and Beaumont, 1925; Baker and Heald, 1932 and others) from time to time. The 'black-tip' disease of mango fruit recently reported (Das Gupta and Verma, 1939) seems to be frequent in orchards situated near brick kilns. If the gases emanating from the kilns have any direct effect on the fruits, the role of lenticels as channels of gaseous exchange may not be insignificant. It is for these reasons that the present investigation has been carried out.

The term 'lenticel' was originally adopted by De Candolle for such structures occurring on stems of flowering plants. In structural details the lenticels of fruits and stems differ. The layer of cambium cells invariably present in lenticels of the stem is usually lacking in the mango and other fruits. Nevertheless the same term may be applied in the fruits as a matter of convenience. Clements (1935) has used the term 'lenticel' in the case of pomes of *Pyrus Malus* and has rightly pointed out that the use by some workers of the names like fruit 'spots', 'dots', or 'pits' to denote lenticels of fruits involves a certain amount of confusion. These terms are liable to be misunderstood since the same expressions have often been employed by plant pathologists to indicate symptoms of certain fruit diseases. The pome being morphologically a stem structure the use of the term 'lenticel', in the author's opinion, is still more justified. In the case of true fruits such as mangoes, the essential structure of the lenticels is the same as in pomes of *Pyrus Malus* and therefore in the present paper the term 'lenticel' has been retained and refers to the small spots seen on the skin of healthy mango fruits. Lenticels without the characteristic cambium, designated as 'ventilating pits', have been reported in petioles of members of the Cyatheaceæ and the Marattiaceæ by Haning (ex. Haberlandt, 1914), but for reasons stated above Haning's terminology need not be followed for fruits.

MATERIAL AND METHODS

Three varieties of mango fruits, namely *safeda*, *dasehri* and *bambai*, were selected for study. Fruits in various stages of development starting from the youngest stage to the very mature one were collected from

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Begum Bagh and Sikandarbagh orchards, Lucknow, during the months of March, April and May 1941. Epidermal peelings of these fruits were made in order to study the stomatal and lenticel structures in surface view. The peelings were made by treating small pieces of fruits with strong nitric acid and potassium chlorate for 12–24 hours, depending upon the stage of maturity of fruits. The soft part including the entire mesocarp was thus macerated and by shaking the treated pieces in water in a test-tube the epidermal peelings separated neatly from the rest of the tissue. The peelings were then treated with strong ammonia solution, washed in water and mounted in glycerine. Pieces of fruits were also embedded in paraffin and microtomed to help in the study of the structure of mature and developing, as well as open and closed lenticels. The open and closed lenticels were ascertained by the technique followed by Clements (1935) for *Pyrus Malus*. The fresh fruits were dipped in a solution of methyl blue at room temperature (30° C.) and later transferred to a cold chamber at 15° C. for about 24 hours. In so doing the skin of the fruit contracts and the lenticels are subjected to a mild expansion without being ruptured. The coloured solution passes through the open lenticels which show a halo of the dye while the closed lenticels remain uncoloured.

The number of lenticels, closed and open, was ascertained in all the three varieties of fruits by direct counts.

Structure of Lenticels

The lenticels appear as minute specks on the skin of fruits. The spots are of different sizes in the three varieties of mango. They are bigger in *bambai* than in *safeda* and *dasehri*, the last having the smallest spots among the three. The apparent colour of the lenticels ranges from light to dark brown and is often brick red as in *safeda*.

In surface view, as seen in peelings of the epidermis of mature fruits under the microscope, the lenticel region shows an opening of an irregular form surrounded by much divided subsidiary (of stomatal origin) and epidermal cells which radiate from the opening in all directions (Pl. VI, Figs. 1, 2, 3).

In vertical sections the lenticel apertures are seen as breaks in the epidermis (Text-figs. 1, 2 and 3). Below the opening a number of hypodermal cells are loosely packed, the number depending upon the size of the lenticels. These hypodermal cells are filled with a tannin-like substance to which the apparent colour of the lenticels is due. In fruits having larger lenticels the hypodermal cells seem to radiate from the .opening. This is due to the pulling force acting on the hypodermal cells during the stretching of the epidermis as the fruit matures and grows in size. Below the hypodermal cells are ordinary parenchymatous cells of the pericarp. Unlike lenticels of stems of angiosperms, the .lenticels in mango fruits do not show any sign of development of a cambium under the loosely packed hypodermal cells, and in this respect differ from them. As already stated, it is the absence of this cambium that should evoke difference of opinion as to the validity and correctness of the use of the term 'lenticel' (primarily employed to describe the lenticels of stems of angiosperms) for such structures on fruits.

DEVELOPMENT OF LENTICELS

Lenticels which occur invariably on the skin of mature mango fruits are, however, not percievable when the fruits are very young. An examination of the epidermal peelings of fruits of this age reveals that the skin bears a number of stomata, but no lenticels are found.

The stomatal apparatus consists of two large guard cells with a substomatal chamber below and a stomatal pore above (Text-figs. 4–11). The guard cells have their walls thin on the side away from the pore while round the latter the wall is very thick, forming a thickened poral rim. The poral rim is much more clear in surface view when the stomatal pore is closed than when it is open. In the region of the poral rim the guard cells have projecting cuticular ridges which in a section appear as horn-like cuticular projections from the guard cells and partly overarch the stomatal pore (Text-figs. 9–11).

Subsidiary cells which usually accompany guard cells in many angiosperms seem to develop, in the case of mango fruits, after the guard cells are fully distinct. In very young stages of the fruit the guard cells are surrounded by ordinary epidermal cells (Text-figs. 4–8), but later on an enlarged cell appears on the lateral side of each guard cell. Thus on the side of each guard cell appears a lateral subsidiary cell. No polar subsidiary cells have been found to develop in this case. As the fruits grow the lateral subsidiary cells begin to overarch the guard cells (Text-figs. 14–17). Each subsidiary cell then divides transversely into three or sometimes four cells, which further overarch the guard cells (Text-figs. 18–20, and Pl. VI, Figs. 4–6). This type of subsidiary cells has also been reported in some angiosperms (Bandulska, 1924, 1926) and the Benettitales (Florin, 1933). In vertical sections of the stomata at this stage the guard cells are seen to be superposed by the subsidiary cells (Text-figs. 12, 13).

The first step in the development of lenticels is the permanent opening of the guard cells. The lateral subsidiary cells further divide and completely overarch the guard cells leaving only the stomatal pore. In surface view these cells lie round the pore (Text-figs. 21–25). By further division the subsidiary cells merge into the adjoining epidermal cells and in so doing they spread radially from the stomatal pore, probably under the force caused by the stretching of the epidermis as the fruits mature (Pl. VI, Figs. 1–3). Due to the stretching of the epidermis on all sides, the pore is widened and forms the lenticel aperture. At the same time the hypodermal cells below the substomatal chamber undergo change, under the same influence, which creates intercellular spaces among them (Text-figs. 1–3). The hypodermal cells get a deposit of tannin-like substance to which the colour of the lenticels, in surface view, is due. The number of hypodermal cells taking part depends on the size of the lenticels. Thus the least number is involved in *dasehri*, where the lenticels are the smallest, and the largest in *bambai* where the lenticels are the biggest among the three varieties studied. During these changes the guard cells of the stomata remain intact for quite a long time and can be recognised particularly in surface view. The shape and form of the guard cells do not, however, remain the same
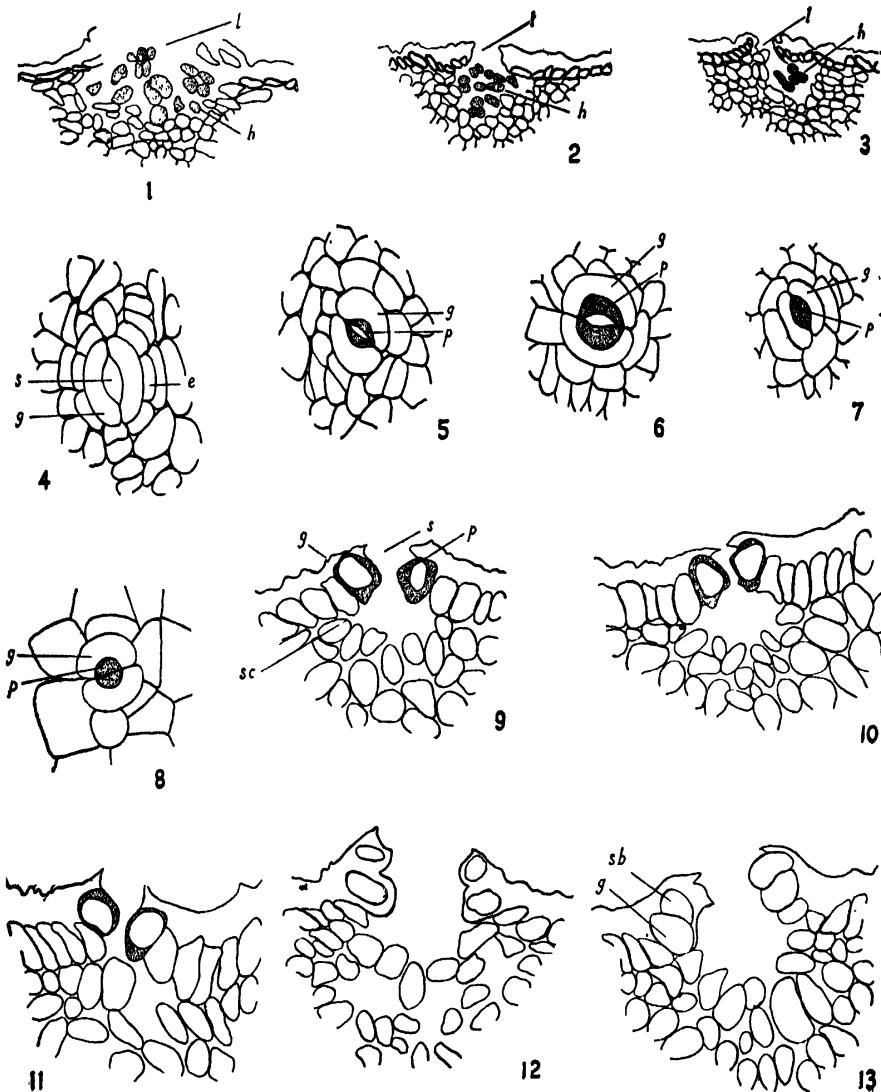
Text-Figs. 1–13.—Figs. 1–3. Lenticels in vertical sections. *l*, lenticel aperture ; *h*, loosely packed hypodermal cells. 1. *bambai;* 2. *safeda;* 3. *dasehri.* ×94. Figs. 4–8. Stomata in surface view. *s*, stomatal pore ; *g*, guard cells ; *e*, epidermal cell ; *p*, poral rim. 4–5. *bambai;* 6–7. *safeda;* 8. *dasehri.* ×375. Figs. 9–11. Same in vertical sections. *sc*, substomatal chamber. 9. *bambai;* 10. *safeda;* 11. *dasehri.* ×375.   Figs. 12–13. Same when guard cells are overarched by subsidiary cells (*sb*). 12. *bambai;* 13. *safeda.* ×375.

when seen in a vertical section. It is to be pointed out here that all the stomata in a fruit do not develop into lenticels. In such cases the

guard cells shrivel and the pore becomes permanently closed and as the fruit enlarges they can be seen lying somewhat disorganised among the developing lenticels.
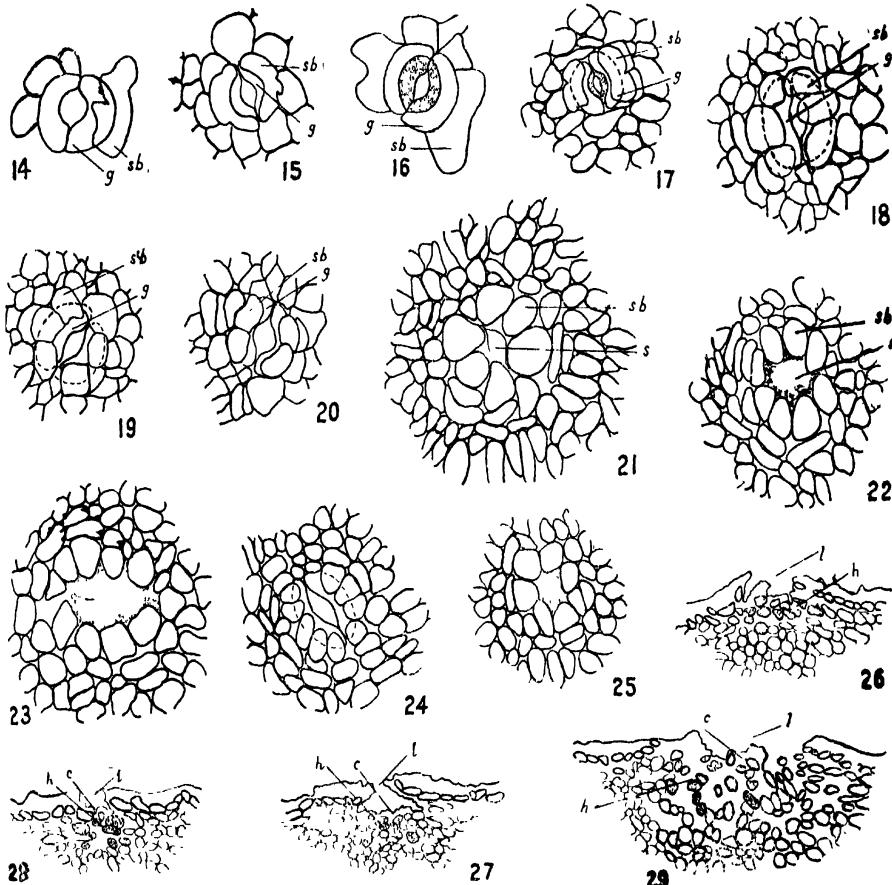
Text-Figs. 14-29.—Figs. 14-25. Stages in development of lenticels from stomata, as seen in surface view. *s*, stomatal pore ; *g*, guard cell ; *sb*, subsidiary cell. 14, 21, 22. *bambai.* ×375. 17, 18. *bambai.* ×240. 15, 19. *safeda.* ×240. 23. *safeda.* ×375. 16. *dasehri.* ×375. 20, 24, 25. *dasehri.* ×240. Figs. 26-28. Vertical sections of closed lenticels. *l*, lenticel aperture ; *h*, hypodermal cells ; *c*, cuticle. 26. *bambai ;* 27. *safeda ;* 28. *dasehri.* ×84. Fig. 29. *bambai.* A partially closed lenticel in vertical section. ×84.

As the fruits grow some of the lenticels become closed, while the rest remain open. Closed and open lenticels were ascertained by the technique followed by Clements (1935) for apples and described above. The closing of the lenticels has been found to occur by the development of a layer of cuticle on the outermost layer of the hypodermal cells. The extent of development of this layer of cuticle determines

the complete or partial closure of the lenticels. In fully closed lenticels, the cuticle develops over the whole length of the hypodermal cells (Text-figs. 26–28), while in partly closed ones the cuticle does not cover some of the hypodermal cells (Text-fig. 29). Lenticels, in which the cuticle does not at all cover the hypodermal cells, are regarded as open (Text-figs. 1–3).

DISTRIBUTION OF LENTICELS IN MATURE FRUITS

It was thought desirable to note the distribution of lenticels, closed and open, on the surface of the fruits. For this purpose a piece of graph paper divided in square centimeters was spread over the surface of the fruit and by pricking a needle through the paper, the four corners of the squares were marked off on the skin of the fruit. These squares were then completed on the skin of the fruit with ink and direct counts of lenticels in these areas were made under a magnifying lens. Counts were made for the total number of lenticels, both open and closed, per unit area in the basal and apical halves of the fruit, and also for open and closed lenticels separately, in any region of the fruit. The unit area taken was 9 sq. cms. in order to cover sufficient surface. Twelve fruits of each variety were taken and in each fruit different regions of the halves were covered in counting the number of lenticels.

Table showing the number and distribution of lenticels per 9 sq. cms. in mature fruits

Variety of Fruit	Total number of lenticels both open and closed		Number of open lenticels	Number of closed lenticels
	Basal half	Apical half		
safeda ..	190–198	240–248	25–36	160–172
dasehri ..	190–196	260–266	35–40	150–215
bambai ..	70–79	100–120	65–81	7–11

It will be noticed from the above table that the total number of lenticels in *bambai* is the least, while there is no marked difference between *safeda* and *dasehri*. It is also evident that lenticels are more numerous in the apical half of the fruit than in the basal half. In *safeda* and *dasehri* more of the lenticels are closed than open, while the reverse is the case in *bambai*.

CONCLUSION

It is evident from the foregoing observations that the skin of the mature mango fruit has numerous lenticels which look like tiny spots to the naked eye. In structure, there is a general correspondence with the lenticels found on stems of angiosperms, although there are differences in details. In the first instance, the fruit lenticels are comparatively very small in size, the number of hypodermal cells

involved being just a few. The hypodermal cells do not characteristically radiate from the lenticel opening in all cases as is the case with stem lenticels. Another important difference is the absence of the cambium cells below the hypodermal cells in fruit lenticels, while in lenticels of stem the cambium is conspicuously developed (Haberlandt, 1914). It is the absence of this cambium that has led to the discussion on the correctness or otherwise of the use of the term 'lenticel' in the case of fruits.

It has been found that the lenticels in mango fruit develop from stomata as also observed by several other workers (Zschokke, 1897, Tetley, 1930, Clements, 1935) for apples. In the development of the lenticel from the stoma it has been observed that the lateral subsidiary cells of the guard cells play an important role. The subsidiary cells, having divided transversely into several cells, gradually overarch the guard cells leaving only the stomatal pore which is now permanently open. It is the stomatal pore that forms the lenticel aperture which widens up rather irregularly under the stretching force of the enlarging fruit. Under the same influence the subsidiary cells along with the epidermal cells radiate from the pore in all directions, giving the lenticel region a characterstic appearance in surface view. At the same time some of the hypodermal cells below the substomatal chamber get loosely arranged and develop deposits of tannin which gives the brown colour to the lenticel. The overarching of guard cells by subsidiary cells has been observed before in leaves of angiosperms (Bandulska, 1924, 1926) and the Benettitales (Florin, 1933), but has not been reported for fruits so far in author's knowledge. In mature fruits some of the lenticels become closed by the development of cuticle on the outermost layer of hypodermal cells, while others remain open.

A study of the number and distribution of lenticels shows that certain varieties (*safeda* and *dasehri*) have more lenticels per unit area than others (*bambai*), and also that they are more in the apical half of the fruit than in the basal half. As regards the open and the closed lenticels it has been found, that in *safeda* and *dasehri* more of the lenticels are closed than open, while reverse is the case in *bambai*.

SUMMARY

The structure and development of lenticels in mango fruits have been described. The lenticels have been found to develop from stomata which alone are present in young fruits. The stomata become permanently open and the guard cells are gradually overarched by lateral subsidiary cells which divide and merge into the cells of the epidermis. As a result of the stretching, to which the epidermal cells of the enlarging fruit are subjected, the stomatal pore also enlarges, forming the lenticel aperture, from which the subsidiary and the epidermal cells seem to radiate. The hypodermal cells below the stomatal pore also come under the influence of stretching and become loosely arranged in rows radiating from the pore. Their walls become brown. The cambium, a usual feature below the hypodermal cells in stem lenticels, is absent in the lenticels of mango fruits. In the

mature fruit some of the lenticels become fully or partly closed by a complete or partial development of a layer of cuticle over the hypodermal cells.

All the stomata do not develop into lenticels. Lenticels are more numerous in the apical half of the fruit than in the basal half.

ACKNOWLEDGMENT

I wish to express my deep gratitude to Dr. S. N. Das Gupta for his help and guidance during the course of this investigation and to Prof. B. Sahni for permitting the use of his library.

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EXPLANATION OF PLATE VI

Figs. 1–3. Lenticels in surface view. (1) *bambai*, (2) *safeda*, (3) *dasehri*. All ×200.

Figs. 4–6. Surface view of stomata overarched by the lateral subsidiary cells. (4) *bambai*, (5) *safeda*, (6) *dasehri*. All ×200.
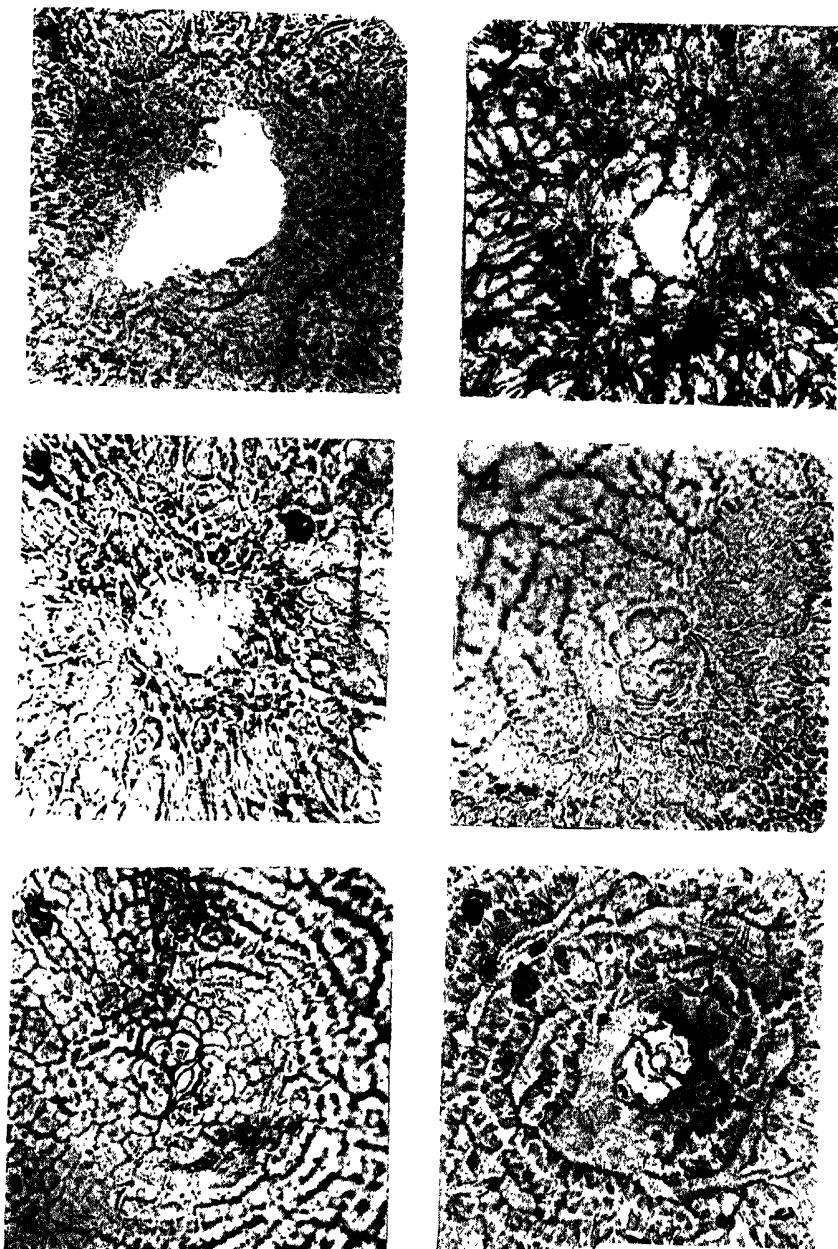
A REVISION OF THE INDO-BURMESE SPECIES OF *LINDERNIA* ALLIONI

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THE species properly referable to this genus have received different treatments from different taxonomists and have been placed under 2, 3 or even 4 separate genera, *viz.*, *Lindernia*, *Vandellia* Brown ex Linn., *Ilysanthes* Raf. and *Bonnaya* Link and Otto. The authors in most cases differ in their opinion in placing the species under these genera and many of them, realising that the line of demarcation is too fine between these artificial genera, have freely confessed their difficulties in trying to keep them separate. The character, on which the taxonomists who are in favour of keeping the four genera separate mostly depend, is the number of fertile stamens, 4 in some cases, and 2 in others with 2 staminodes. These staminodes show transitional stages and vary in different specimens of the same species. Cases are recorded where species which should have all the stamens fertile, have in some specimens two barren stamens.

Lindernia and *Vandellia*, both having 4 fertile stamens, are treated as separate by Linnæaus, Urban and few others, but have been combined by Bentham* and many others, and the generic name used, was *Vandellia* Linn., *Lindernia* All., or *Pyxidarsia* Hall. *Ilysanthes* and *Bonnaya* have 2 fertile stamens and 2 staminodes. In *Ilysanthes*, the fertile stamens are posterior and the staminodes anterior, while in *Bonnaya* the arrangement is just the reverse. For this reason, these two have been treated as distinct by Bentham, J. D. Hooker, Urban and a few others but have been united by Wettstein, Otto Kuntze, Hemsley and Skan, Hutchinson and Dalzell and most of the subsequent authors.

Thus we find that most of the taxonomists agree that there should be 2 genera instead of 4. Haines in his *Botany of Bihar and Orissa* (pp. 630–635), has also reduced the 4 genera into 2 but in a different manner. He has united *Bonnaya* with *Vandellia* and *Ilysanthes* with *Lindernia*. In reducing *Bonnaya* to *Vandellia* he makes the following remarks :—*"The genus *Bonnaya* differs from *Vandellia* as defined in the *Genera Plantarum* of Bentham and Hooker only by two of the stamens not bearing fertile anthers. But in view of the following considerations the separation of these two genera on this character alone appears

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* Bentham at first treated *Vandellia* as distinct from *Lindernia* in DC. Prod. **X**, 418, but later combined the two under *Vandellia* in his *Scroph. Ind.* and in *Genera Plantarum*.

to me artificial and untenable. In *V. molluginoides*, Hooker found in
some specimens 2 filiform staminodes hooked near the top, and I have
a specimen of *V. Crustacea* in which the two anterior filaments appear
to be without anthers. Some species of *Vandellia* can only be sepa-
rated from species of *Bonnaya* by this one sexual character, *e.g.*, forms
of *V. angustifolia* and *V. verbenæfolia*. Finally the character
of the barren stamens themselves differs and shows transitional
stages. In *Bonnaya verbenalfolia*, the anterior stamens in some
specimens bear anthers but these are small and unfertile. In
B. veronicæfolia there are no anthers, but the filaments are long and
curved or hooked as in the case of some *Vandellia molluginodes*. In
Bonnaya brachiata the barren stamens are short pubescent scales. I
have therefore united the two genera."

For the separation of his *Vandellia* and *Lindernia* Haines depends
only on one point, *i.e.*, the nervation of leaves as suggested by J. D.
Hooker. It would have been more appropriate for him to go one
step further and combine the four into a single genus, but this was to
be done by Pennell, who in his Scroph. of East Temperate North
America (*Monographs, Academy of Natural Sciences of Philadelphia*,
No. 1, 1935) reduced *Vandellia, Ilysanthes* and *Bonnaya* into *Lindernia*.
Pennell's remarks in this connection are worth quoting : "By the union
of the four-anthered *Lindernia* All. and *Vandellia* L. with the two—
anthered *Ilysanthes* Raf. and *Bonnaya* Link and Otto. is formed a large
and natural genus. It is characterised by the remarkably uniform
corolla (with narrow posterior lip much shorter than the widely
spreading anterior lip), by similar curiously recurving anterior filaments
(the proximal portion of each projecting as if it were an appendage and
the filament forked although actually the process is formed by the sharp
inbending of the filament) and by similar septicidal dehiscence of the
capsule (that nearly always leaves the entire septum persisting as a
median plate)."

The total number of species under *Lindernia* in its new and ampli-
fied form would be about 70, of which 28 are found within the bounda-
ries of India and Burma. These are enumerated below. A modified
description of the genus with a key to the species found in our area is
also given.

LINDERNIA ALLIONI

Herbs, usually annual, slender, creeping or erect, branched,
glabrous or pubescent, often growing in marshy places. Leaves
opposite, entire or toothed, penninerved or parallel-nerved. Flowers
small, sessile or pedunculate, solitary in the axils of leaves in terminal
racemes, bracteolate, often deflexed in fruit. Calyx 5-toothed or 5-
partite with linear segments, scarcely imbricating. Corolla-tube
cylindric or somewhat enlarged above ; upper lip erect, broad, concave,
emarginate or sharply 2-fid, lower lip larger, spreading, with 3 broad,
subequal lobes. Stamens 4, all perfect or the posticous or the anticous
pair reduced to staminodes ; filaments filiform, the posticous pair
affixed to the corolla tube, the anticous pair affixed to the throat, with
a tooth-like or subulate appendage at the base ; anthers subcoherent

or coherent ; cells divaricate, often confluent at the apex. Style bilamellate at the apex. Ovules numerous in each cell. Capsule globose, ovoid, oblong to linear, septicidal ; valves slender, entire. Seeds numerous, foveolate, rugose.

A. Capsule about equalling the calyx or shorter.

  B. Leaves penninerved.
    C. Flowering calyx cleft to the middle or less.
      D. Pedicels about 4 times as long as the fruiting calyx   ..   ..   ..   crustacea.
      D. Pedicels twice as long as the fruiting calyx or shorter.
        E. Leaf blade 1·5 cm. long or less, glabrous. molluginoides.
        E. Leaf blade 2–2·5 cm. long, hairy on both surfaces ..   ..   Hookeri.
    C. Flowering calyx cleft to the base or nearly so.
      D. Flowers 3–4 mm. long.
        E. Non-succulent herbs, leaves petioled   ..   elata.
        E. Succulent herbs, leaves (at least the upper) sessile.
          F. Glabrous ; sepals shorter than capsule.   multiflora.
          F. Pubescent with spreading hairs ; sepals longer than capsule   ..   ..   hirsuta.
      D. Flowers 8–12 mm. long.
        E. Herbs not succulent ; racemes, sub-umbellate, or flowers solitary.
          F. Leaves 1–1·5 cm. long, sparsely hairy.
            G. Fruiting calyx equalling the capsule.   hirta.
            G. Fruiting calyx twice as long as the capsule   ..   ..   ..   laxa.
          F. Leaves 2·5–3·5 cm. long villous on both surfaces   ..   ..   mollis.
        E. Herbs succulent ; racemes elongate   ..   punctata.
  B. Leaves parallel-nerved.
    C. Perfect stamens 4   ..   ..   ..   pyxidaria.
    C. Perfect stamens 2.
      D. Erect herb, very small and slender ; corolla thrice as long as calyx   ..   ..   minima.
      D. Diffuse or creeping herb ; corolla twice as long as calyx   ..   ..   ..   rotundifolia.

A. Capsule twice as long as calyx or longer.

  B. Perfect stamens 4.
    C. Flowering calyx cleft to the middle.
      D. Flowers pedicelled   ..   ..   ..   numularifolia.
      D. Flowers sessile   ..   ..   ..   sessiliflora.
    C. Flowering calyx cleft almost to the base.
      D. Leaves ovate, shortly petioled   ..   ..   cordifolia.
      D. Leaves linear or linear-lanceolate, sessile   angustifolia.

B. Perfect stamens 2.
  C. Leaves parallel-nerved.
    D. Corolla 3–4 times as long as calyx ..   hyssopioides.
    D. Corolla twice as long as calyx or shorter. parviflora.
  C. Leaves penninerved.
    D. Corolla white or red.
      E. Staminodes present.
        F. Staminodes hairy.
          G. Leaves sessile, corolla 6–7 mm.
            long, fruit 12–15 mm. long ..   ciliata.
          G. Leaves petioled, corolla 18–20 mm.
            long, fruit 25–30 mm. long ..   ruelloides.
        F. Staminodes glabrous.
          G. Leaves very sharply spinous-serrate,
            teeth 1–1·5 mm. apart ..   bractioides.
          G. Leaves shallowy serrate, teeth about
            3 mm. apart ..   ..   .. quinqueloba.
      E. Staminodes absent ..   ..   .. estaminodiosa.
    D. Corolla blue or violet.
      E. Corolla 12 mm. or more long.
        F. Capsule linear-subulate, leaves broadly
          elliptic to ovate-oblong ..   .. anagallis.
        F. Capsule narrowly cylindric ; leaves
          linear or narrowly lanceolate ..   verbenæfolia.
      E. Corolla 6 mm. long or shorter.
        F. Leaves distantly and shallowly toothed,
          oblong   .. oppositifolia.
        F. Leaves entire or nearly so, linear ..   tenuifolia.

1. **Lindernia crustacea** (Linn.) F. Muell. Cens. Austral. Pl. p. 97;
    Pennell in Acad. Nat. Sc. Phil. Monogr. 5, (1943), p. 29.
    Caparia crustacea Linn. Mant. 87.
    Vandellia crustacea Bth. Scroph. Ind. 35, and in DC. Prod. X,
    p. 413. Hk. f. Fl. Brit. Ind. IV, 279.
    Torenia varians Roxb. Fl. Ind. 111, p. 96.
    Throughout India, up to 1,600 m. in the Himalayas ; tropics
    of Old World, introduced into tropical parts of America.

2. **L. molluginoides** (Bth.) Wettst. in Nat. Pflanzen. f. iv. 3b.,
    p. 80.
    Vandellia molluginoides Bth. Scroph. Ind., p. 35 and in DC.
    Prod. X., p. 413. Hk. f. *loc. cit.* 279.
    Burma.

3. **L. Hookeri.** (Cl.) Wettst. *loc. cit.*, p. 79 ; Pennell *loc. cit.* 39.
    Vandellia Hookeri Cl. ex. Hk. f. *loc. cit.*, 280.
    V. stemonoides Prain Bengal Pl., p. 762 ; Haines Bot. Bihar and
    Orissa, 631 ; non Miq.
    Bihar, Chotanagpur, N. Bengal, Khasia Mts., Pegu.
  L. Hookeri subsp. **kumaunensis** Pennell, *loc. cit.*, p. 30.
    Kumaon.

4. **L. elata** (Bth.) Wettst. *loc. cit.*, p. 79.
   Vandellia elata Bth. Scroph. Ind., p. 36 and in DC. Prod. X,
   p. 414 ; Hk. f., *loc. cit.*, 280.
   Burma.

5. **L. multiflora** (Roxb.) Mukerjee Comb. nov.
   Vandellia multiflora G. Don. Gen. Syst. IV, 549 ; Bth. in DC.
   Prod. X, p. 414. Hk. f. *loc. cit.*, 280.
   V. erecta Bth. Scroph. Ind., p. 36, in part.
   Torenia multiflora Roxb. Fl. Ind. 111, p. 96.
   Bengal.

6. **L. hirsuta** (Bth.) Wettst. in Nat. Pflanzen. f. IV, 36, p. 79.
   Vandellia hirsuta Bth. Scroph. Ind., p. 36, and in DC. Prod. X.,
   p. 44. Hk. f. Fl. Brit. Ind. IV, 280.
   S. India, Sikkim Terai and Bengal to Burma ; Ceylon and
   eastwards to Phillippine Islands.

7. **L. hirta** (Cham. and Schl.) Mukerjee comb. nov.
   L. scabra (Bth.) Wettst. *loc. cit.*, 79.
   Torenia hirta Cham. and Schl. in Linnæ 11, p. 571.
   Vandellia scabra Bth. Scroph. Ind., p. 36, and in DC. Prod. X,
   p. 414, Hk, f. *loc. cit.*, p. 281.
   Columnea minuta Roxb. Fl. Ind. 111, p. 98.
   Southern and eastern parts of India ; S. Africa, Mada-
   gascar and tropical parts of Asia.

8. **L. laxa** (Bth.) Mukerjee comb. nov.
   Vandellia laxa Bth. Scroph. Ind., p. 36 and in DC. Prod. X., 414,
   Blatter and Hallb. in Bomb. Nat. Hist. Soc. XXV (1918), p. 416.
   V. scabra var laxa Hk. f. *loc. cit.*, p. 281.
   Vingrola-Konkan ; High wavy mts.-Madura Dist., Guindy
   Madras (Prov.).

9. **L. mollis** (Bth.) Wettst., *loc. cit.*, p. 79.
   Vandellia mollis Bth. Scroph. Ind., p. 37 and in DC. Prod. X.,
   p. 414, Hk. f., *loc. cit.*, p. 281.
   Sikkim, Assam, Burma, S. China.

10. **L. punctata** (Prain) Mukerjee comb. nov.
    Vandellia punctata Prain in Journ. Roy. As. Soc. Bengal LXXII
    (1903), p. 19.
    Shan Hills, Port, Stedman, Taungyi.

11. **L. pyxidaria** All. in Misc. Taurin 3 (1766) 178, tab. 5 ; Linn.
    Mant. Pl. 2 ; (1771) 252 ; Pennell in Acad. Nat. Sc. Phil.
    Monograph, 5 (1943), p. 25.
    L. erecta Botanii ?
    Vandellia erecta Bth. Scroph. Ind. 36, and in DC. Prod. X.,
    p. 415, Hk. f. *loc. cit.*, 281.
    Gratiola integrifolia Roxb. Fl. Ind. i, 137.
    Throughout India upto 1,700 m. in the Himalayas ; east-
    wards to Polynesia, and westwards to Europe.

12. **L. minima** (Bth.) Mukerjee comb. nov.
    Ilysanthes minima Bth. in DC. Prod. X, p. 420, Hk. f. Fl. Brit.
    Ind. IV, p. 284.
       S. India.

13. **L. rotundifolia** (Linn.) Mukerjee comb. nov.
    Gratiola rotundifolia Linn. Mant., p. 274 ; Roxb. Cor. Pl. iii,
    3, t. 204, and Fl. Ind., p. 137.
    Ilysanthes rotundifolia Bth. in DC. Prod. X, p. 420 ; Hk. f.
    loc. cit., 254.
       S. India, Ceylon, Mauritius and Madagascar.

14. **L. numularifolia** (Don) Wettst. in Nat. Pflanzenf. IV, 3b, p. 97,
    Pennell in Acad. Nat. Soc. Phil. Monogr. 5, (1943), p. 29.
    Vandellia numularifolia Don. Prod. Fl. Nep., p. 86 ; Bth. in DC.
    Prod. X, p. 416 ; Hk. f. *loc. cit.*, p. 282.
       Subtropical Himalayas from Kashmir to the Mishmi Hills,
    S. India, Chotanagpur, Assam and hills of Burma.

15. **L. sessliflora** (Bth.) Wettst. *loc. cit.*, p. 79.
    Bonnaya micrantha Blat. and Hallb. in Journ. Bomb. Nat. Hist.
    Soc., XXV (1918), p. 417.
    Vandellia sessiliflora Bth. Scroph. Ind., p. 37 and in DC. Prod.
    X, p. 416, Hk. f. *loc. cit.*, 282.
       Subtropical Himalayas, Assam, Burma, S. Ind ia.

16. **L. cordifolia** (Colsm.) Merrill Enum, Phillp. Pl. III, p. 437 ;
    Pennell, *loc. cit.*, p. 30.
    Gratiola cordifolia Colsm. Prod. Desc. Grat., p. 15.
    Vandellia pedunculata Benth Scroph. Ind. 37 and in DC. Prod
    X, 416, Hk f., *loc cit.* 282.
    Vandellia cordifolia G. Don, Gen. Syst. IV, p. 549 ; Haines
    Bot. Bihar and Orissa, p. 633.
    V. cerastoides Collet and Hemsl. in Journ. Linn. Soc., Vol.
    XXVIII (1890), p. 100.
       Throughout India, Ceylon, Malaya and eastwards to
    Australia.

17. **L. angustifolia** (Bth.) Wettst., *loc cit.*, 79, Pennell, *loc. cit.*, p. 31.
    Vandellia angustifolia Bth. Scroph. Ind., p. 37, and in DC. Prod.
    X, p. 417, Hk, f., *loc. cit.*, p. 282.
    V. verbenaefolia Haines Bot. Bih. and Orissa, p. 634, in part.
       Subtropical Himalayas, Kumaon, Nepal, Sikkim, Chota-
    nagpur, Assam, Burma.

18. **L. hyssopioides** (Bth.) Haines, *loc. cit.*, p. 635.
    Ilysanthes hyssopioides Bth. in DC. Prod. X, p. 419 ; Hk. f.,
    loc. cit., p. 283.
       S. India, Chotanagpur (Sarguja), Assam, Burma (?) ;
    Ceylon, Malaya, China.

19. **L. parviflora** (Roxb.) Haines., *loc. cit.*, p. 635, Pennell, *loc. cit.*,
    p. 29.
    Gratiola parviflora, Roxb. Cor. Pl. III, p. 3, t. 204 and in Fl. Ind.,
    I, p. 140.

Ilysanthes parviflora Bth. in DC. Prod. X, p. 419, and Scroph. Ind. 34 ; Hk. f., *loc. cit.*, 283.

Throughout India upto 1800 m. in the Himalayas ; Siam, Malaya peninsula ; Trop. Africa.

20. **L. ciliata** (Colsm.) Pennell in Journ. Arn. Arb. Vol. 24 (1943), p. 253 ; et. in Monog. Acad. Nat. Soc. Phil. No. 5, p. 32.
Gratiola ciliata Colsm. Prod. Desc. Grat., p. 14.
Bonnaya brachiata Link and Otto, Icon. Pl. Select. 25, t. 11 ; Hk. f., *loc. cit.*, p. 284.
Vandellia brachiata Haines, *loc. cit.*, p. 632.

Throughout India, up to 1,600 m., in the Himalayas ; Ceylon, Malaya and eastwards to the Philippine Islands.

21. **L. ruelloides** (Colsm.) Mukerjee comb. nov.
Gratiola ruelloides Colsm. Prod. Desc. Gratiola 12, Roxb. Fl. Ind., p. 140.
Bonnaya reptans Spr. Syst. 1, p. 410 ; Hk. f., *loc cit.*, p. 284.
Ilysanthes reptans Urban Berl. Deutsch. Bot. Ges. 11, p. 436.
L. ruelloides O. Ktze Gen. Pl., p. 462.

Nepal, Sikkim, Assam and Burma ; South Ind a, Java, Phillipine Islands.

22. **L. bracteoides** (Blat. and Hallb.) Mukerjee Comb. nov.
Bonnaya bractoides Blat. and Hallb. in Journ. Bomb. Nat. Hist. Soc. XXV (1916), p. 416.

Common in Abu Mts.

23. **L. quinqueloba** (Blat. and Hallb.) Mukerjee comb. nov.
Bonnaya quinqueloba Blatt. and Hallb. in Journ. Bomb. Nat. Hist. Soc. XXV (1918), p. 417.

24. **L. estaminodiosa** (Blat. and Hallb.) Mukerjee comb. nov.
Bonnaya estaminodiosa Blat. and Hallb. in Journ. Bomb. Nat. Hist. Soc. XXV (1918), p. 416.

Mahim, Bombay Island.

25. **L. anagallis** (Burm.) Pennell in Journ. Arn. Arb. Vol. 24 (1943), p. 252.
Ruellia anagallis Burm. Fl. Ind., p. 135.
Bonnaya veronicæfolia Spr. Syst. 1. 14 ; Hk. f. *loc. cit.* 285.
Vandellia veronicæjolia Haines, *loc. cit.*, 633.
L. antipoda Alston in Trim. Handb. Fl. Ceylon VI ; suppl., p. 214.
Throughout India ; eastwards to the Philippine Islands.
Var grandiflora (Spr.) Mukerjee comb. nov.
B. grandiflora Spr., *loc. cit.*, p. 41.
B. veronicæfolia var *grandiflora* Hk. f., *loc. cit.*, 285.
Throughout India.

26. **L. Verbenæfolia** (Colsm.) Pennell, *loc. cit.*, p. 131.
Gratiola verbenæfolia Colsm. Prod. Desc. Grat., p. 8·
Bonnaya verbenæjolia Bth. in DC. Prod. X, p. 421.
B. veronicæjolia var. verbenaefolia Hk. f., *loc. cit.*, p. 295.
Vandellia verbenæfolia Haines, *loc. cit.*, p. 634.

Upper Gangetic Plain, Bengal, Burma, S. E. Asia.

27. **L. oppositifolia** (Linn.) Mukerjee comb. nov.

   Gratiola oppositifolia Linn. Sp. Pl. ed. Willd., Vol. I, p. 105,
      Roxb. Cor. Pl. II, p. 30, t. 155.

   Bonnaya oppositifolia Spr. Syst. 1, p. 41, Benth. in DC. Prod. X,
      p. 421 ; Hk. f., *loc. cit.*, 286.

   Vandellia oppositifolia Haines Bot. Bihar and Orissa, p. 634.

   Ilysanthes oppositifolia Urban in Berl. Deutsch. Bot. Ges. II
      (1884), p. 435.

      S. India, Manbhum.

28. **L. tenuifolia** (Vahl.) Alston in Trim. Fl. Ceylon, VI, Suppl.,
   p. 214.

   Gratiola tenuifolia Vahl. Enum. 1, p. 95.

   Bonnaya tenuifolia Spr. Syst. 1, p. 42 ; Bth. in DC. Prod. X,
      p. 422 ; Hk. f., *loc. cit.* 286.

   Ilysanthes tenuifolia Haines Bot. Bihar and Orissa, p. 634.

      Bengal to Burma, S. India ; Ceylon and China.

AN ANATOMICAL STUDY OF *TILIACORA ACUMINATA* MIERS.

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1. Introduction

Tiliacora acuminata (Lam.) Miers. (= *T. racemosa* Colebr.) is a diœcious evergreen climber of the Menispermaceæ, which is known to occur in various parts of India, Burma, Ceylon and Malaya (Brandis, 1874).

The plant is usually seen near hedges and bushy clumps and it frequently climbs over forest trees. The young shoots are green but the older ones are covered with a thin brownish layer of cork. The lenticels are few in number and elongated along the axis of the stem with their margins slightly raised above the general surface. A cross-section of an old stem presents an extremely abnormal appearance, for, instead of a single ring of vascular tissue as is met with in most dicotyledons, we find here a series of concentric rings or large arcs of bundles separated by tangential bands of parenchyma, while in between the bundles of each ring lie the wide interfascicular rays (Figs. 1, 2*a*). Eighteen such rings were counted at the base of a stem, about 8·8 cm. in diameter. Owing to the abundance of fibrous cells in the wood, the stems show a high degree of flexibility and are consequently used in many places for thatching and basket work.

• The full grown leaves are 16·5 to 19·0 cm. by 9 to 11 cm. in size, 3- to 5-nerved at the base, ovate, acuminate and cordate, truncate or rounded at the base with undulate margins, and glabrous except on the lower side of the midrib. The petiole is 2·5 to 3·5 cm. in length with its base somewhat flattened and twisted so as to serve as a hook for helping the plant in attaching itself to its support.

The root system consists of a woody tap-root with many branches. The anomaly seen in the stem is also present here (Figs. 2*b* and *c*) and 7 rings of vascular tissue were seen in a root about 4·2 cm. thick. According to Roxburgh (1832, Vol. 3, p. 816) the root is used as a cure for snake-bite.

2. Previous Work

A reference to the works of Solereder (1908) and Pfeiffer (1926) shows that anomalous secondary growth has so far been noted in the family Menispermaceæ in the stems and roots of *Abuta, Chondrodendron Cissampelos, Clypea* and *Cocculus* and in the stems only of *Anamirta, Anomospermum, Chasmanthera, Hyperbæna, Jateorhiza, Menispermum,*

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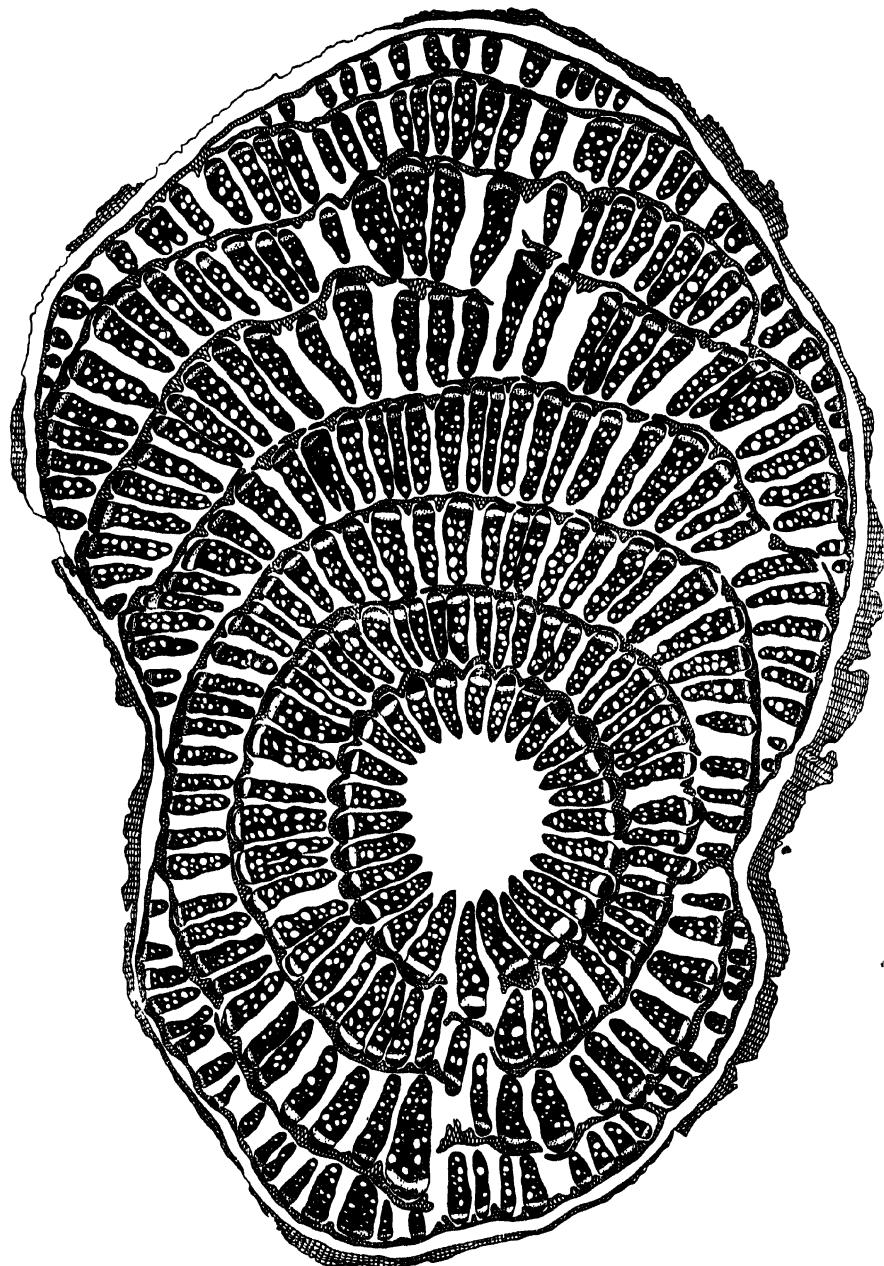
Fig. 1.   An outline diagram of t.s. of stem showing anomalous
secondary growth. × 9.

Pachygone, Pericampylus and *Stephania*. Recently Santos (1931) described it in the stems of *Anamirta cocculus* and *Archangelisia flava* and Maheshwari (1935) has reported it in the stem of *Tiliacora*. No detailed work on the anatomy of the last named plant has, however, yet appeared and this study was undertaken in the hope that it might lead to a clearer idea of the origin of the supernumerary cambial rings which form such a characteristic feature of the stem and root in several plants of the family.

3. MATERIAL AND METHODS

The greater part of the material used in this study was collected locally and fixed in formalin-acetic-alcohol. This was dehydrated, infiltrated and imbedded in paraffin according to the usual methods. Older pieces of the stem and root were first treated with dilute HF. Section were cut between 12 and 25 μ and stained with Safranin and Fast Green. In a few cases Bismarck Brown and Iron-hæmatoxylin were also used for comparison. The oldest stems and roots were cut fresh on a sliding microtome. Mounts of macerated material were also prepared for a study of the shapes and characteristics of the individual cells and the strip method of Priestley, Scott and Malins (1933) was used to make some preparations of the tissues adjacent to the primary cambium[1].

4. THE STEM

Primary Structure.—A cross-section of the stem is roughly circular with some undulations in the younger stages which smoothen out afterwards. The *epidermis* consists of a layer of moderately cutinised cells with occasional stomata. Epidermal hairs are few, occurring only in the youngest regions. They are invariably 2-celled with a short stalk cell which is thick-walled and urn-shaped and a terminal cell which is long, slender and thin-walled. The deposition of cutin is usually seen only on the outer walls but occasionally it extends even to the radial and inner walls of some epidermal cells.

The *cortex* consists of 3–4 layers of collenchymatous cells followed by 4–10 layers of more or less tangentially elongated parenchyma. Chloroplasts are present in both but are more numerous in the outer layers, particularly in the substomatal portions. Solitary thick-walled cells of a fibrous nature are also seen in the cortex but only occasionally and without any definite plan in their arrangement or distribution. "Bitter principle sacs" such as have been noted in *Tinospora* by Santos (1928) were seen in only a few sections.

An *endodermis* is not clearly distinguishable. Many cells of the innermost layer of the cortex which are in contact with the band of sclerenchyma contain rectangular or rhomboidal crystals of calcium oxalate. Due to the lack of a distinct endodermal layer it is not possible to set off the tissues of the stele from the cortex with any

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[1] The designation " primary cambium " is used here to distinguish the cambium of the normal ring of vascular bundles from the subsequently formed " secondary " or " extrafascicular " meristems.

certainty, but by analogy with other menispermaceous stems (see Santos, 1928, 1931), the crescent-shaped arches of sclerenchymatous cells (Fig. 3) which lie outside the vascular ring may be said to belong to the pericycle. The outer of these cells are small and thick-walled and have a narrow lumen while the inner are larger and do not show such a pronounced thickening of their walls. In macerated material the fibres are seen as long and tapering cells with simple slit-like pits on their walls. Next to the sclerenchyma comes a thin-walled parenchymatous tissue, the inner pericycle, composed of 3–5 layers of closely packed polygonal cells which become greatly compressed and flattened in older stages. It is of interest to note that although originally there is a continuous cyclinder of pericyclic fibres, this later becomes ruptured as a result of the increase in girth of the stem ; the inter-fascicular parenchyma cells intrude however into the gaps and become converted into stone cells thus repairing the broken cylinder.

The *vascular bundles* vary in number from about 20 to 40 and are separated by the medullary rays which are usually 4 to 8 cells wide and consist of radially elongated cells with small intercellular spaces. An increase in the number of bundles may be brought about either by the splitting of the original bundles owing to the formation of secondary vascular rays, or by the production of secondary strands in the inter-fascicular region. It is frequently seen that the bundles on one side of the stem are larger than those on the opposite side, thus giving it an eccentric appearance which becomes still more pronounced in subsequent stages.

The *pith* occupies a large proportion of the space in a young stem. The cells are large and spherical with prominent inter-cellular spaces at the angles. The peripheral cells are more thick-walled than those in the centre and form a sort of perimedullary zone as is seen in many other dicotyledons (Eames and Macdaniels, 1925, p. 99). Starch grains and calcium oxalate crystals are common.

Secondary Growth.—At a very early stage a fascicular cambium differentiates in each bundle and gives rise to the secondary tissues in the normal manner. There is however no definite inter-fascicular cambium, although some stray tangential divisions are occasionally seen in the cells of the medullary rays. A glance at Figs. 3 and 4 will show that the sclerenchymatous cells of the pericycle invaginate so deeply into the rays that the formation of a continuous cambial ring is hardly possible.

As a result of the activity of the fascicular cambium the primary phloem and afterwards even the older secondary phloem cells become crushed and obliterated to form a densely staining cap over the vascular bundles (Fig. 4). It is worthy of note that the sieve plates are not transverse to the longitudinal axis of the sieve tubes but lie at angle of about 45°.

The secondary xylem consists of vessels, fibre-tracheids and wood parenchyma. The vessels are often large enough to be made out with the naked eye. They occur either singly or in groups of two to four. The vessel elements have simple rounded perforations and

some of them are provided with tails at one or both ends.   Their walls show alternately arranged bordered pits with slit-like apertures which are just included in the border.   Fibre-tracheids are abundant and have thick walls with numerous bordered pits having slit-like apertures. The xylem parenchyma cells may be short or elongated and have simple pits with rounded, oval or slit-like openings.   The ray cells have simple pits except when they are in contact with vessels or fibre-tracheids in which case the pits are bordered.   In tangential   sections and strip preparations the rays appear as large more or less homogeneous boat-shaped areas whose lateral cells are particularly rich in calcium oxalate crystals.   Short thin-walled "disjunctive parenchyma"[2] cells are also seen although   only occasionally.   These are wider, having simple rounded or oval pits and short tubular processes.

The older stems usually become eccentric owing to more active growth on one side (Fig. 1).   According to Priestley and Tong (1927), Haberlandt (1914, p. 676) and others, such eccentricity in growth may be caused by gravity, mechanical strain, light, moisture, nutrition, temperature, wind, etc., having an unequal influence on the two opposite sides of the stem or root of plants.   I have not been able to determine the cause of the eccentricity but the lower side is usually the one which shows more active growth in *Tiliacora* (*cf.* Maheshwari, 1930, on *Boerhaavia*).

Cork formation begins   only   after a fair amount of secondary growth has taken place.   The subepidermal cells undergo some radial elongation followed by tangential divisions which result in the demarcation of the phellogen.   The cork cells produced on the outside are more or less rectangular in cross-section and lie in distinct radial rows. The phelloderm, which is produced on the inside, is very narrow and with increase of secondary growth the shape and arrangement of its cells become distorted.   Crystals of calcium oxalate are of frequent occurrence and some of the cells become converted into stone cells.

Anomalous Secondary   Growth.—The primary cambium becomes inactive after some time (Fig. 4).   When this happens, the parenchymatous cells (Fig. 5) lying just outside the sclerenchymatous bands show a radial elongation (Fig. 6) and   soon begin to undergo some periclinal divisions, as a result of which the inner cortex becomes converted into a meristematic zone composed of about 4 to 10 layers of cells.   A few of the outermost layers of this tissue usually remain undifferentiated and afterwards give rise to another ring of vascular tissue as we shall see later.   The next 1 to 3 layers give rise to a ring of stone cells[3] (Fig. 7) and in the remaining portion of the meristem further periclinal and anticlinal divisions now take place at a number of points resulting in the differentiation of groups of phloem cells (Fig. 8) followed

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[2] This name has now replaced the "conjugate parenchyma" of older authors (see Record, 1933, p. 9).

[3] The radial series cannot always be traced outside the stone cells due to the distortion brought about by the inward pressure of the periderm and the outward pressure of the secondary vascular tissue.

internally by some xylem elements (Fig. 9). Some intervening cells between the xylem and phloem continue to retain their meristematic character and form the intrafascicular cambium. As mentioned before, the anomalous growth at first starts in short segments, but it extends laterally to form fairly large arcs of secondary vascular tissue which abut upon the inner ring of bundles (Fig. 10).

Secondary activity in the newly formed ring of bundles is short-lived but another meristem soon arises from the residual cells of the previous one which had been left undifferentiated at its outer edge. The differentiation of stone cells and vascular tissues in this tertiary ring takes place in a manner similar to that already described for the second ring. The subsequent rings originate from the outer cells of their predecessor as in the case of the second and third rings. The production of these supernumerary rings continues for a long time, resulting in the marked polycyclic condition already noted in the beginning of this paper.

The vascular bundles of the anomalous rings are very similar to those of the primary ring except that the former lack all primary xylem and phloem and that the primary ring has on its outside an undulate band composed mainly of fibrous cells while those formed later are overarched by one to three layers of stone cells. Owing to the presence of these sclerenchymatous elements outside every ring of vascular tissue, the pluriseriate nature of the stem is very clear even to the naked eye. The xylem vessels are often found to contain tyloses which are at first thin and bladder-like but later become pitted.

5. THE LEAF

Petiole.—A cross-section of the petiole presents a more or less broad crescent-shaped outline. The cuticular deposit on the epidermis extends even to the radial walls of its cells as in the case of the stem. Epidermal hairs are of the same kind as seen in the stem and are continued on the lower side of the lamina. Next to the epidermis come 3 to 6 layers of compactly arranged collenchymatous cells with chloroplasts. In the inner cortex, which consists mainly of parenchymatous cells, there are also some isolated sclerenchymatous elements with thick walls and a broad lumen. Three leaf traces enter the petiole, all more or less completely surrounded by fibrous sheaths. The median trace divides immediately into three bundles and the other two also branch and anastomose, thus resulting in a total of about 8 to 12 strands arranged in a semicircle. This differs from the condition in *Anamirta, Menispermum, Cocculus* (Solereder, 1908, p. 41) and *Tinospora* (Santos, 1928) where the bundles are arranged in a circle and the petiole has a more or less rounded outline in a cross-section.

The pith consists of fairly large spherical cells with small intercellular spaces. Many of the cells contain calcium oxalate dust.

Lamina.—The leaf is bifacial. In a cross-section of the lamina the upper epidermis is seen to consist of tabular or squarish cells whose outer walls are slightly convex but not so heavily cutinised as those of the stem or petiole. The mesophyll consists of a single layer of palisade

tissue followed by the irregularly shaped cells of the spongy parenchyma which are interspersed with large air spaces. Each vein consists of a small vascular bundle surrounded by a fibrous sheath which extends both inward and downward as to some close to the epidermal layers. The cells of the sheath occasionally show small rhomboidal crystals of calcium oxalate. In some plants of the family, Solereder (1908, p. 815) and Santos (1928) record the presence of large rhomboidal crystals in the epidermal layers, which serve as a 'regular armour' : but I was unable to find them in either the lower or the upper epidermis of *Tiliacora acuminata.* Stomata were seen only on the lower surface.

The midrib is conspicuous on account of the local convexity of the leaf tissues in this region. As seen in a transverse section the epidermal cells on both sides of the midrib are usually polygonal and more thick-walled than those on the sides. Just below the epidermis, on either side of the lamina, there are 1 to 3 layers of collenchymatous cells those on the lower side being somewhat larger. They frequently contain crystals of calcium oxalate. Next to the collenchymatous tissue is the parenchyma with occasional stone cells which are more frequent in the basal part of the leaf.

The number of vascular bundles composing the midrib depends on the size of the leaf and the distance from the base at which the section is taken. The largest number (about 13) is seen at the base of the lamina, from where they pass off towards the right and left until there is only one bundle left in the distal portion of the midrib.

6. THE ROOT

Primary Structure.—A cross-section of the young root is more or less circular in outline and shows the usual piliferous layer, cortex and stele. Suberisation of the outer walls of the epidermis begins soon after the root hairs have ceased to function and extends even to layer below it. The cortex consists of about 3 to 6 layers of large polygonal cells which are often full of fungal hyphæ[4] (Fig. 11). The innermost layer forms an indistinct endodermis, sometimes easily recognisable by the Casparian strips on its radial walls which are more easily seen opposite to the primary phloem strands. Inside the endodermis there is a single layer of pericycle. The stele is usually diarch but sometimes triarch, and the primary xylem elements meet in the centre to form a "xylem plate" (Figs. 11, 13). It is only in a few roots that some small parenchymatous cells resembling those of a pith were seen in the centre but even these become lignified in later stages. In some cases they were found to contain starch grains. Some of the cells immediately outside the primary phloem and perhaps belonging to it become lignified and form fibres.

Secondary Structure.—Secondary growth begins early by the usual differentiation of cambial segments below the primary phloem bundles

and their subsequent extension resulting in a complete cambial cylinder. The secondary xylem elements extend so close to the primary xylem that in later stages it is difficult to demarcate the latter. Opposite to the primary xylem groups the cambium cuts off only parenchymatous tissue resulting in two (three, when the root is triarch) broad medullary rays, which divide the secondary vascular tissues into large semicircular segments. Very soon additional vascular rays originate inside each segment which is consequently split up into separate bundles of which as many as 15 may be seen in later stages.

The structure of the secondary vascular bundles is very similar to those of the stem. Tyloses are frequently present in the vessels. As secondary growth proceeds, the primary phloem cells become flattened and crushed, and a one to two-layered ring of sclerenchymatous elements appears at the periphery of the secondary vascular bundles.

Very soon after the vascular cambium has begun its activity a phellogen differentiates in the pericycle. It cuts off 7–10 layers of phellem towards the outside but much less of phelloderm. Here and there inside the former are seen some thick-walled elements which have a mechanical function. With the development of the cork, all the tissues outside it (*i.e.*, the cortex and the epidermis) die and are sloughed off.

Anomalous Secondary Growth.—The abnormal growth starts in the roots from the pericyclic cells internal to the phellogen, which first elongate radially and then divide periclinally to form a zone of meristematic cells, 8 to 10 cells wide. As in the stem, isolated patches of phloem and xylem separated by a cambium are differentiated inside it. On the outside of the meristematic zone there differentiates a layer or two of sclerenchymatous cells which often contain calcium oxalate crystals and further outward (as in the stem) a few layers of the meristem are left over to form the precursors of the next anomalous ring. Other rings (Fig. 12) are produced similarly in centrifugal succession from the residual cells of the previous meristem left over immediately outside the layer of stone cells.

7. DISCUSSION

Anomalous thickening in plants, characterised by the production of additional complete or partial extrafascicular cambial cylinders, is observed in lianes as well as non-lianes. As regards the modes of origin of the anomalous rings, Solereder (1908, p. 1164–65) divides the dicotyledonous families into two groups :—

(i) Those in which the secondary meristem arises either in the inner cortex or occasionally even in the endodermis : Capparideæ, Caryophylleæ, Compositæ, Cucurbitaceæ, Menispermaceæ, Plumbagineæ Umbelliferæ and Verbenaceæ.

(ii) Those in which the place of origin lies deeper, either in the pericycle or even in the phloem of the original vascular ring : Amaranthaceæ, Ampelidaceæ, Bignoniaceæ, Buxaceæ, Cæsalpinieæ,

Candolleaceæ (?), Capparideæ, Chenopodiaceæ, Compositæ, Convolvulaceæ, Cucurbitaceæ, Dilleniaceæ, Euphorbiaceæ, Ficoideæ, Hippocrateaceæ, Icacineæ, Illecebraceæ, Labiatæ, Loranthaceæ, Nyctaginaceæ, Olacineæ, Phytolaccaceæ, Plumbagineæ, Polygaleæ, Rhamneæ (?), Rubiaceæ and Sapindaceæ.

In addition to the above families Pfeiffer (1926) mentions the same abnormality in the Acanthaceæ, Vochysiaceæ, Cruciferæ, and with some doubt in the Loganiaceæ, and Stylidiaceæ. A similar anomaly is met with among the Gymnosperms in the Cycadaceæ and Gnetaceæ.

As regards the Menispermaceæ, Maheu (quoted in Solereder, 1908, p. 818) reported that the anomalous rings may originate in any of the following tissues :—

(a) Cortex (*Menispermum*).

(b) Endodermis (*Abuta rufescens, Chondrodendron tomentosum, C. platyphyllum* and *Cocculus laurifolius*).

(c) Pericycle (*Cocculus læba, Cissampelos pareira*).

(d) The region immediately external to the phloem of the normal ring of bundles (*Abuta selloana, Anomospermum grandifolium, Cocculus platyphyllus* and *Cissampelos mouritiana*).

Pfeiffer (1926, p. 164) remarks however that Maheu's observations, which were made almost entirely on herbarium material, are not quite reliable, and suggests further work on fresh material. The only recent paper on the subject is by Santos (1931) who found that in the stems of *Archangelisia flava* and *Anamirta cocculus* the abnormal rings are formed by successively produced cambial layers differentiating in the primary cortex. Should this be so and the cambia continue to arise from the cortical cells in centrifugal succession, a time will eventually arrive when the cortex would be used up altogether. In *Tiliacora acuminata* only about 12–14 layers of cells are found in the primary cortex, but a cross-section of a thick stem already showed as many as 17 anomalous rings and there was no indication that the abnormal growth had come to an end. It was this that prompted me to make a closer study of the origin of the anomaly and compare my observations with those of other recent authors. In the stem of *Boerhaavia diffusa* (Maheshwari, 1930) and the root of *Beta vulgaris* (Artschwager, 1926), the supplementary rings of vascular tissue do not arise *de novo*, but their origin has been traced back to the normal cambial ring. Joshi (1931, 1937) supported this in the case of *Alternanthera sessilis* and other Amarantaceæ and Chenopodiaceæ and there seems to be no doubt that the supernumerary vascular rings found in the Centrospermales are in direct lineage with the first extrafascicular cambium.

My observations on *Tiliacora acuminata* correspond with those of the above authors and are at variance with the results obtained by Santos (1931). Briefly, there is at first a single ring of vascular bundles in the stem, separated from each other by broad medullary rays. The cambium in these bundles becomes inactive after a time and a new extrafascicular cambium arises in the cells lying immediately outside the pericycle. This gives rise to a broad zone of meristematic cells

a few layers of which lying at the periphery remain undifferentiated for the present ; the next 1–3 layers give rise to stone cells and the rest undergo further divisions to produce a ring of secondary vascular bundles. Now, after a brief period of secondary growth in these bundles, the two or three layers of undifferentiated cells left over outside the ring of stone cells begin active divisions and behave in the same way as the first meristem. This process repeats itself several times, giving a pluriseriate character to the stem. The same anomaly occurs in the root with this difference that here the secondary cambium and the phellogen both originate in the pericycle.

It is worthy of note that two different types of secondary growth occur in the family Menispermaceæ. A few genera like *Tinospora* (Santos, 1928) show only the normal type, while others (see list on p. 2) are characterised by the formation of successive rings of centrifugally differentiated vascular bundles. A study of the published data on the habit of the plants seems to indicate that the difference is not related to the environment.

Whether this condition is the result of parallel development or whether one of the two conditions is derived from the other is therefore difficult to say in the present state of our knowledge of the morphology and cytology of the plants belonging to this family.

8. SUMMARY

1. A cross-section of the young stem of *Tiliacora acuminata* shows a normal ring of vascular bundles separated by broad medullary rays. An interfascicular cambium is inactive or absent. The pericycle consists of a ring of sclerenchymatous elements made up of arcs of fibres capping each vascular bundle. A well-differentiated endodermis is absent.

2. Older stems show a series of concentric rings of vascular bundles (18 rings were counted in a stem 8·8 cm. thick), separated by wide rays and tangential bands of stone cells and thin-walled parenchyma.

3. Sections cut at different levels of the stem show that after a while the normal fascicular cambia of the primary ring of bundles become inactive and an extrafascicular meristem originates from the cortical cells lying immediately outside the pericycle. A few of the outermost layers of this meristem remain undifferentiated, the next 1–3 layers give rise to stone cells, and the rest differentiate into a secondary ring of vascular bundles separated by broad rays of parenchymatous cells. After a time the activity of the cambia in these bundles also ceases and a second meristem arises from the undifferentiated cells of the first one left over outside the stone cells. This process is repeated so as to give rise to a large number of anomalous rings of vascular bundles arising in centrifugal succession.

4. There is a well-marked eccentricity in the stems, the lower side showing greater growth and a larger number of anomalous rings (or their segments) than the upper.

5. Three leaf-traces enter the petiole, but they further split and anastomose in their course so that there is no constancy in their number and 8 to 12 bundles may be seen in a cross-section of the petiole.

6. The lamina shows the usual structure of a mesophytic dicotyledonous leaf with the stomata confined to the lower surface. The midrib has about a dozen bundles at the base but these pass out right and left and ultimately only a single bundle is seen at its distal end.

7. The root is usually diarch but sometimes triarch. The cortical cells are often full of fungal hyphæ. Cork formation takes place in the pericycle, and after the production of the first and normal ring of secondary vascular tissues a new cambium arises from the pericyclic cells internal to the periderm and gives rise to an additional ring of vascular bundles outside the first one. As in the stem, there is a centrifugal succession of such rings of vascular tissue which gives a pluriseriate appearance to the mature roots.

8. Crystals of calcium oxalate are found in all parts of the plant.

9. ACKNOWLEDGMENTS

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EXPLANATION OF PLATES

Plate VII

Fig. 2a. Transversely cut surface of a thick stem showing the supernumerary rings of vascular tissues ; *b & c*. Cross and longitudinal sections of the root to show the same anomaly. ×½.

Fig. 3. T.s. of portion of a young stem. ×70.

Fig. 4. T.s. of a part of stem showing the approximate stage after which anomalous growth starts. ×70.

Plate VIII

Fig. 5. T.s. of a portion of young stem showing the epidermis, cortex, and the sclerenchymatous pericycle. ×290.

Fig. 6. Part of an older stem showing the origin of extra-fascicular cambium. Note the radial elongation and periclinal divisions in the inner cortical cells. ×290.

Fig. 7. The meristematic zone is well established and a band of stone cells is being differentiated at its periphery. Note that a few cells of the meristem are left over at the outer edge of the stone cells. The radial rows are especially clear towards the left. ×220.

Fig. 8. Later stage showing the differentiation of a group of phloem cells from the meristem. ×220.

Fig. 9. Still later stage showing the differentiation of a vascular bundle consisting of both xylem and phloem. ×220.

Fig. 10. Portion of t.s. of a stem showing the inner or normal and the outer or abnormal ring of vascular tissue. ×37.

Plate IX

Fig. 11. T.s. of a young root showing the primary xylem plate. Note fungal hyphæ in the cortical cells. ×212.

Fig. 12. T.s. of an old root showing the anomaly. ×6.

Fig. 13. T.s. of the central portion of an old root showing primary xylem and part of secondary xylem and medullary rays. ×190.
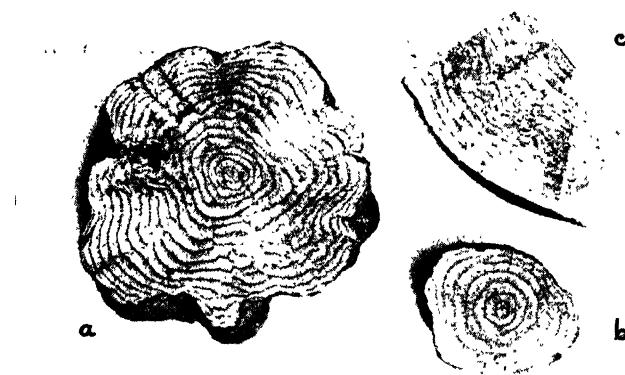
Fig. 2
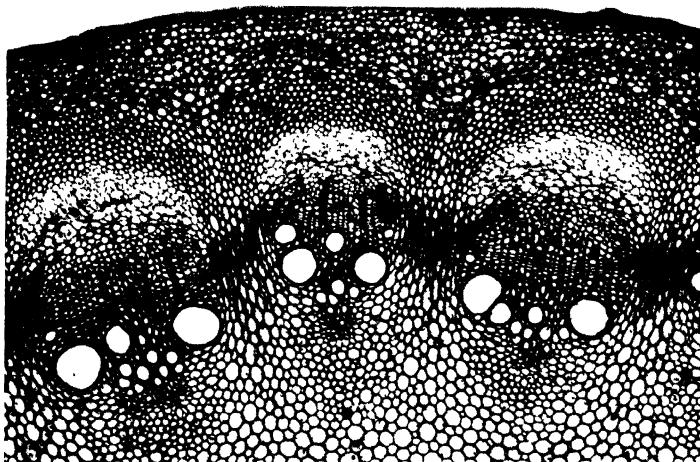
Fig. 3
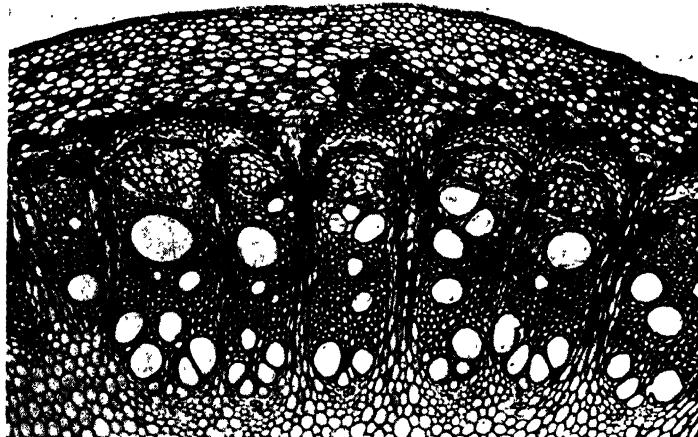
Fig. 4
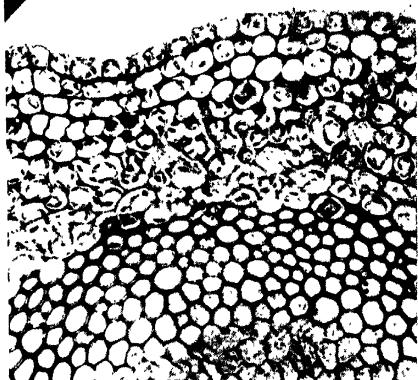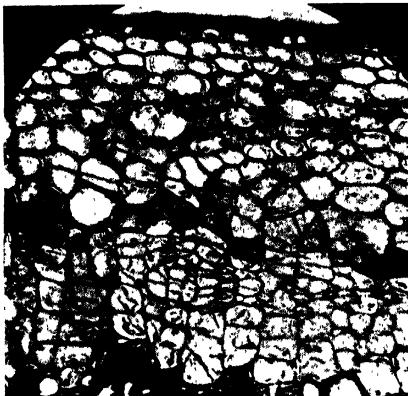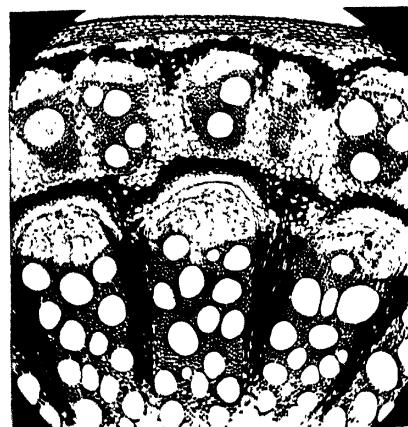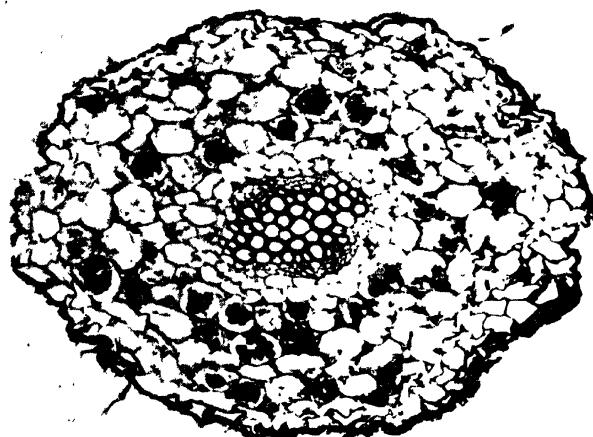
Fig. 11

Fig. 12

Fig. 13

BALWANT SINGH—
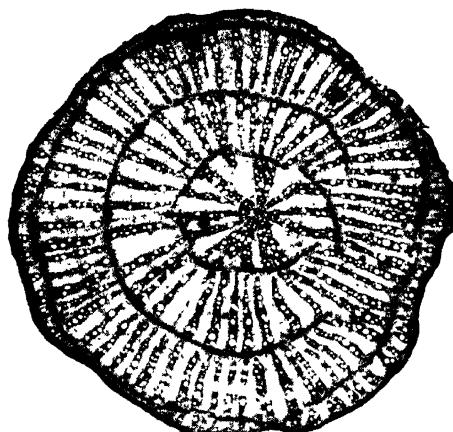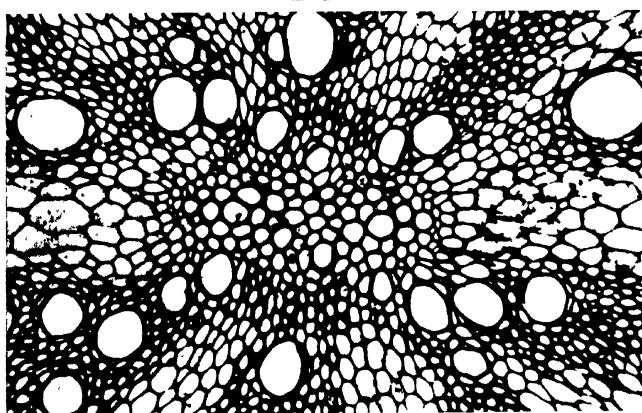
SOME FOSSIL LEAFLETS OF *AESCULUS INDICA* COLEBR. FROM THE KAREWA BEDS AT LAREDURA AND NINGAL NULLAH, PIR PANJAL, KASHMIR

BY G. S. PURI

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INTRODUCTION

THIS paper is based on a few fossil leaflets collected by the author in 1941 from the Lower Karewa deposits of Kashmir (Lower Pleistocene in age), exposed near Laredura (34° 7' N. ; 74° 21' E.), a small village at an altitude of 6,000 ft., about 7½ miles from Baramulla. The bridle path leading to Gulmarg at the seventh mile bifurcates into two narrow paths, one of which turns southwest and winds through a thick shrubby growth of *Parrotia jacquemontiana, Rosa Webbiana, Rubus* spp., etc., in a forest of *Cedrus Deodara* and passes through paddy fields to reach the main huts of the village. The fossiliferous outcrop lies in a steep cliff which is vertically exposed both at top and at the bottom. Our specimens were collected *in situ* from one of the spots lying towards the upper part of the cliff.

The fossil impressions are embedded in a thickly laminated clay, dirty black or yellowish in colour, and splits crudely along the planes of bedding.

DESCRIPTION

Order : Sapindales

Family : Sapindaceæ

The family is represented in the Karewa Flora by a few leaflet fragments, which are referred to a single species of the genus *Aesculus ;*

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all the specimens are well preserved impressions. Living plants of this species have compound leaves with 5–7 leaflets arising from a common stalk in a palmate manner. The two lower and outer leaflets are the smallest in size and the one in the middle is the largest. Fossil leaflets of all sizes have been found, but they are all detached and fragmentary specimens.

Aesculus indica Colebr.

Figs. 1–5

The specimens described below are two leaflet fragments, one of which (Fig. 1) is almost complete and measures 4·5 inches long by 2·2 inches in the broadest part, which lies a little above the middle. The lamina, which is oblong-lanceolate in shape, gradually narrows down from the middle into cuneate base. It is broadly acute or short acuminate at the apex. The margins are closely sharp serrate ; teeth are small and sharply pointed. Fig. 3 is a natural size photograph of another fragment which, though badly preserved as regards details of venation, illustrates a smaller leaflet. The two leaflets figured here show a good deal of difference in size.

The venation is pinnate and reticulate with a tendency to form small loops under and close to the margins. A strong midrib arises from the base and runs in the lamina slightly thinning out in the upper part. 11–12 secondaries which are almost half as thick as the midirb, arise from the latter on either side in an alternate manner at open angles. The laterals in Fig. 1 curve upwards and inwards to from small shallow loops a little beneath and close to the margins. The loops are not well preserved and do not show up conspicuously even in living leaflets (Figs. 4, 5). The tertiary ribs are small and thin; they arise from the two opposite laterals and run in the area enclosed by them ; and anastomose variously to form large meshes of different shapes and sizes, seen clearly in Fig. 2, which represents a part of a leaflet shown in Fig. 1 enlarged to five diameters. There is a finer reticulation, which consists of a network of small, rectangular or polygonal meshes (Fig. 2).

The fossil leaflets are identical in all respects with modern leaflets of *Aesculus indica* Colebr. (Figs. 4, 5).

Number of Specimens.—Five.

Occurrence.—Spot No. 2 and Spot No. 5 Laredura at 6,000 ft., and Ningal Nullah at 9,000 ft., and locality No. 1, Ningal Nullah at 9,500 ft., Pir Panjal, Kashmir.

Collection.—G. S. Puri, 1941.

Registered Nos. of figured Specimens.—Pl. X, Figs. 1, 2 = L 662/2; Pl. X, Fig 3 = L 814/5.

MODERN DISTRIBUTION OF THE SPECIES

The genus *Aesculus* is included by Hooker in the Sapindaceæ in his *Flora of British India*, but Rendle describes it under the family

Hippocastaneæ, which contains 2 genera and 18 species, mainly represented at the present time in the United States of America, although they are distributed throughout the north temperate zone.

A very large number of modern species of *Aesculus* occur in North America ; it may be interesting to point out that the species growing on the Atlantic and Pacific sides of the continent are quite different ; one species extends northwards into Canada. In Europe, we have only one species (*A. Hippocastanum*), which is a wild tree of the Albanian and Northern Greece mountains. The Old World is not rich in horse-chestnuts; Japan having two species, while there is only one in Northern China ; and we have two in the Himalayas. The Himalayan species are large handsome deciduous trees, one of which *A. indica* has been found in a fossil state in the Karewas and its modern distribution in India is given below :

The Indian horse-chestnut grows in the Western Himalayas extending westwards from Nepal at an altitude of 4,000–9,000 ft.; it usually grows in moist shady ravines and prefers northerly aspects of hills, which are comparatively cooler. It is often gregarious along moist gorges and grows plentifully in steep ravines and on hill-sides. In Hazara it is fairly common, occurring with *Juglans regia, Acer* spp., *Prunus* spp. and other broad-leaved trees together with coniferous species, namely, *Abies Webbiana, Taxus baccata, Picea* sp., *Cedrus Deodora,* and *Pinus excelsa.*

The species is fairly common in Kashmir and occurs within a radius of one quarter of a mile of the fossiliferous locality ; it is represented both on the Himalayan slopes and the northern slopes of the Pir Panjal Range at Gurez, Jhelum Valley, Kamraj, Keran, Kishtwar, Marwa Dachhan, Muzaffarabad, Ramban, Sindh Valley and Udhampur. In the regions adjoining Kashmir the species occurs in Kafirstan at 7,000–8,000 ft., Chitral at 7,500 ft., Kagan Valley at 9,000 ft. ; and in the Murree Hills it is associated with *Quercus dilatata, Q. incana, Prunus* sp., *Acer* sp., *Pinus excelsa, Taxus baccata,* etc. Eastwards, it extends to the Parbatti Valley (Kulu at 7,500 ft.), Chamba State, Kangra, Simla Hills, Mussoorie, Tehri Garhwal, Kumaon, Naini-Tal and Almora.

In the "western oak-fir forests of Garhwal Himalayas"*Aesculus indica* is associated with *Quercus semecarpifolia, Q. dilatata, Ulmus Wallichiana, Acer Cæcium, Corylus colurna, Rosa macrophylla, Syringa Emodi, Viburnum* spp., *Taxus baccata, Abies Pindrow, Picea Morinda,* etc. (Champion, 1936, p. 245).

It also occurs according to Champion (*loc. cit.,* pp. 257–58) in the moist temperate forests, *e.g.,* in Dwali (W. Almora division, Kumaon, United Provinces) its associates are *Acer Cæcium. A. pictum, Carpinus viminea, Ulmus Wallichiana, Betula alnoides, Juglans regia, Fraxinus micrantha, Quercus semecarpifolia, Corylus colurna, Cornus macrophylla, Rhus punjabensis, Taxus baccata, Berberis* spp., *Rubus repens,* etc., but it is associated with *Pinus excelsa, Prunus padus, Viburnum fœtens, Ulmus Wallichiana, Acer Cæcium, Juglans regia,* etc., in the northwest Himalayas at Saran (Kagan division, Hazara).

DISCUSSION

The Indian horse-chestnut occurs in the Western Himalayas at the present time in two different floristic formations, namely (i) in the Kashmir Valley it occurs with *Juglans regia*, *Rhamnus purpurea*, *Populus ciliata*, *Salix Wallichiana*, *Pinus excelsa*, *Abies Webbiana*, etc., and (ii) at another place it is usually associated with conifers and with oaks and other broad-leaved species characteristic of the Western Himalayan rain forests.

· From the available evidence concerning the Karewa flora it seems that *Aeculus indica* during the Early Pleistocene existed with oaks, elms, *Betula*, etc., at one place (Laredura) while its common associates at another place (Ningal Nullah) were willows, cherries, poplars, walnut, etc., etc.

According to Troup (1921, Vol. I, p. 227) the climatic conditions which are most congenial for the growth of *Aesculus indica* under natural habitats include a rainfall varying from 40–100 inches or more ; an absolute maximum shade temperatures between 80° and 102° F. and an absolute minimum varying from 25°–10° F. From this it may be conjectured that the Kashmir Valley during the Early Pleistocene may have had at Laredura and Ningal Nullah climatic conditions somewhat corresponding to the above.

SUMMARY

1. Some leaflets of *Aesculus indica* Colebr. collected by the author from the Lower Karewa deposits (Pleistocene), at Laredura (alt. 6,000 ft.) and Ningal Nullah (alt. 9,000 ft.) are described in detail.

2. The modern distribution of the genus, and of the fossil species is given with special reference to India. At the present time it grows in the Kashmir Valley quite close to the fossiliferous localities and also occurs plentifully at several places in the Kashmir Himalayas and on the Valley slopes of the Pir Panjal Range ; also in the regions adjoining the Kashmir and Jammu territories.

3. The fossil associates of *Aesculus indica* at Laredura and Ningal Nullah are quite different ; and it is pointed out that the Ningal Nullah associates of the species are still existing in the Kashmir Valley, whereas most of the plants, *e.g.*, oaks, *Mallotus*, *Woodfordia*, etc., which were associated with the Indian horse-chestnut at Laredura during the Pleistocene, have now disappeared from the valley proper and the northern slopes of the Pir Panjal Range ; however, the latter occur on the Punjab slopes of the Pir Panjal.

SOME FOSSIL LEAFLETS OF AESCULUS INDICA COLEBR. FROM THE
KAREWA BEDS AT LAREDURA AND NINGAL NULLAH,
PIR PANJAL, KASHMIR

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EXPLANATION OF PLATE

All photographs are from untouched negatives. Figured specimens come from G. S. Puri's collection and are preserved in the Botany Museum, University of Lucknow.

Aesculus indica Colebr.

Fig 1. Fossil leaflet impression. Laredura L 662/3. Nat. size.

Fig. 2 A part of the leaflet enlarged to show meshes of tertiary and finer reticulation ×*Ca.* 5.

Fig. 3 Fossil leaflet impression. Laredura L 814/5. Nat. size.

Fig 4. Modern leaflet, partially rotted before photographing to show venation for comparison with the fossil leaflet (Fig. 4).

Fig. 5. A part of another modern leaflet to show comparison with the fossil leaflet shown in Fig. 1.

STUDIES ON THE EFFECT OF 'SHORT' AND 'LONG DAY' TREATMENT ON THE GROWTH PERIOD AND THE FLOWERING DATES OF DIFFERENT PADDY VARIETIES

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INTRODUCTION

DIFFICULTIES have often been experienced by plant-breeders in effecting crosses between crop varieties differing widely in their flowering dates. This, however, gets more pronounced in a crop like paddy, where the flowering dates differ widely and are more or less fixed for individual varieties or strains, *i.e.*, an early 'Aman' variety shall always flower, under Bihar conditions, in early October whereas the late ones do so at the end of the same month or in early November, when the earlier ones have finished all their flowering and are practically ripe. In such cases, a cross is ordinarily not possible except by utilizing the late tillers of the early variety. This method, however, has its own limitations, specially when the interval between the flowering dates of the varieties to be intercrossed is fairly long. Besides this, the setting in late tillers is usually poor and as such the crosses made at this stage are not always successful. In view of these facts, it was thought that all these difficulties may be successfully solved, if the flowering dates could be suitably altered by increasing or decreasing the 'day length' artificially. This method of altering the time of flowering was initiated by Garner,[4,5,6] who introduced the term photoperiodism to designate the response of plants to the relative length of day and night. Since then many investigators have published results of studies of this phenomenon as it applies to plants. Names of Adams,[1] Evans,[3] Harrington,[7] Ramaley,[8] Shirley,[9] and Tincker[10] may be mentioned as chief workers in the line. Chien-Liang Pan[2] from China published some preliminary results on rice—a crop which does not seem to have been extensively studied and practically no work appears to have been done under Indian conditions. In view of these facts and specially the difficulties encountered by the breeders enumerated above, experiments were taken up to investigate the problem of suitably altering the flowering dates of paddy varieties under Sabour conditions. The results obtained so far are briefly indicated in the present paper.

A. EXPOSING PLANTS TO LONG DAY LENGTH

1. *Material and Method*

In the experiment 30-days old seedlings from one pure strain, 46 BK, were transplanted singly in pots, containing well mixed rice field soil. After a lapse of 30 days from transplanting—*i.e.*, when the

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seedlings had fully established themselves—pots showing fairly uniform growth were selected for experimentation. For each treatment there were six plants.

Constant illumination was given by exposing the plants arranged in a ring to a 200 C.P. Petromax light which was kept in centre uniformly lighted throughout the period of darkness, *i.e.*, from sunset to sunrise. The distance from the Petromax to the plants was 2½ feet.

Plants were exposed to constant light for various periods and at different stages of the growth fully indicated in Table I. After the completion of the treatment plants received only sun light and their subsequent performance was watched carefully and their general growth along with their flowering dates were noted and the results obtained are are given in Table I.

TABLE I

Statement showing the effect of constant illumination on growth and time of flowering of a timely 'fixed' paddy variety

Period of constant illumination	Date on which the plants came to flower	Remarks
1	2	3
Set A— 1. 40 days .. .. (Sept. 1–Oct. 10)	Dec. 12	Growth very poor ; flowering delayed by 46 days
2. 30 days .. .. (Sept. 11–Oct. 10)	Nov. 17–18	Growth poor ; flowering delayed by about 21 days
3. 20 days .. .. (Sept. 21–Oct. 10)	Nov. 7–9	Growth fair ; flowering delayed by about 11 days
4. 10 days .. .. (Oct. 1–10)	Oct. 25–27	Growth practically normal ; no change in the flowering date
Set B— 1. 10 days .. .. (Oct. 1–10)	Oct. 26–27	Growth practically normal ; no change in the flowering dates
2. 20 days .. .. (Aug. 22–Sept. 1)	Nov. 6–9	Growth fair ; flowering delayed by about 10 days
3. 30 days .. .. (Aug. 22–Sept. 20)	Nov. 16–17	Growth fair ; flowering delayed by about 20 days
4. 40 days .. .. (Aug. 22–Sept. 30)	Dec. 8–10	Growth very poor ; flowering delayed by about 42 days
5. In constant light from Sept. 1	..	Plants presented a withered appearance. Treatment was therefore discontinued on Nov. 26
6. Received only the usual sunlight (control)	Oct. 26–27	Normal flowering

2. *Results and their Discussions*

From the above table it is clear that by suitably increasing the length of day, time of flowering can considerably be delayed, in so much so that the plants which received constant illumination continuously for a period extending over 30 days beyond the normal flowering, did not come to flower and ultimately the treatment was abandoned as the plants presented a withered appearance. The above results also show that 10 days of constant illumination does not produce any effect on the flowering dates of paddy whereas a period of 20, 30 and 40 days of constant illumination, irrespective of the stage, earlier (set A) or later (set B) in the growth period, at which it is given, bring about a delay in the flowering dates to the extent of 10, 20 and 45 days respectively, *vide* column 3 of Table I.

After establishing the possibility of delaying the flowering time of paddy by subjecting the plants to increased 'day length', experiments were taken up to study in detail the effect of short 'day length' on flowering time of the various classes of paddy varieties.

B. Exposing Plants to Short Day Length

1. *Material and Method*

The material used in this study consisted of five pure varieties of paddies widely differing in their growth periods, when grown under normal conditions, as indicated below :—

(a) *Periodly fixed paddies*, i.e., '*Aus*'.—

   (i) 'Sathis' takes about 60 days to come to flower from the date of germination, irrespective of the season.

   (ii) 'Aus' No. 28–16–21 : takes about 80 days to come to flower from the date of germination, irrespective of the season.

(b) *Timely fixed paddies*, i.e., '*Amans*'.—

   (iii) Early 'Aman' (115 BK) : flowers between 8–10 Oct.

   (iv) Medium 'Aman' (16 BK) : flowers between 20–22 Oct.

   (v) Late 'Aman' (36 BK) : flowers between 28–30 Oct.

Three seeds of each of these five varieties were sown on 27th May in pots containing well mixed rice field soil. Ultimately in each pot seedlings were thin down to one. Seedlings of the following age of the varieties under experimentation, as indicated below, were included in the test. Age of the seedlings was counted from the date they emerged out of the soil.

Paddy varieties	Age of the seedlings in days
'Sathi'	.. 7, 15 and 30.
'Aus' 28–16–21	.. 7, 15, 30 and 45.
Early 'Aman'	.. 7, 15, 30, 45, 60, 75, 90, 105 and 120.
Medium 'Aman'	.. 7, 15, 30, 45, 60, 75, 90, 105 and 120.
Late 'Aman'	.. 7, 15, 30, 45, 60, 75, 90, 105 and 120.

The 'short days' were given in each case by removing the plants from daylight to a well ventilated dark room at 3 p.m. and 5 p.m. each day.   In the following morning at 5 a.m. they were taken out of the dark room and placed outside in the open along with the control plants.   Each set was replicated 4 times.

In the following order, the seedlings were subjected to the 'short days' on the dates noted against each of them in Table II.

TABLE II

Paddy varieties	Age of the seedlings in days	Dates on which 'short day' treatment was started
1	2	3
1. 'Sathi', 'Aus' and 'Aman'	7	8th June
2. 'Sathi'. 'Aus' and 'Aman'	15	15th June
3. 'Sathi', 'Aus' and 'Aman'	30	30th June
4. 'Aus' and 'Aman'	45	15th July
5. 'Aman'	60	30th July
6. 'Aman'	75	14th August
7. 'Aman'	90	29th August
8 'Aman'	105	13th Sept.
9. 'Aman'	120	28th Sept.

When the plants came to flower the 'short day' treatment was discontinued in each case and the plants were thenceforth kept in open for further growth along with the controls.   Results obtained are fully detailed in Table III.

2. *Results and their Discussions*

It may be seen from Table III that the plants of all the varieties under experimentation in both 3 p.m. and 5 p.m. sets up to 30 days-old seedlings, came to flower at about the same time, end of July, *i.e.*, within 60–63 days of germination.   The actual period of earliness in flowering induced by the 'short day' treatment being dependent on the normal flowering dates of these varieties (columns 7 and 8, Table III) is thus different in the different varieties.   Consequently the 'Aus' variety flowered 19–20 days earlier than the control whereas the early, medium and late 'Aman' varieties flowered earlier by 67–69, 79–81 and 87–89 days than their respective controls.

The 'Sathi' variety did not at all respond to the treatment.   Its 60 days normal period of growth could not be reduced and it thus flowered with the control.

All the varieties in the 45 days set came to flower together on 15th–22nd of August, *i.e.*, about a fortnight later than that of 7, 15 and 30 days sets. In the remaining sets, *i.e.*, 60, 75, 90 and 105, all the varieties under trial flowered together on 1-5th September, 15-18th September, 30th September to 5th October and 14th October to 20th October respectively, *i.e.*,

at fortnightly intervals throughout their growth period. The early paddy in the 105 days set and the medium and late in the 120 days sets, which were near their normal maturity when the light treatment was commenced, did not respond to the treatment and came to flower with the control. The behaviours of the 3 p.m. and 5 p.m sets, in all these cases as well were almost similar. The induced period of early flowering in these sets as the light treatment was given at later dates (column 3, Table III) was correspondingly shorter as fully detailed in columns 7 and 8 of the table under reference.

It may further be seen from Table III that the seedlings of the age of 30 days and over have taken 30–33 days of the light treatment in both the sets of 3 p.m. and 5 p.m. to come to flower earlier than their controls. But the case of 7 and 15 days old seedlings sets is different in this respect. They have, as may be seen, columns 3 and 4 of Table III, taken longer periods of 'short-days', *i.e.*, 53–55 and 45–47 days respectively in both the sets (3 p.m. and 5 p.m.). When we compare these results with that of the seedlings of older age (30 days and over) it seems highly probable that the extra period of 23–25 days in case of 7 days and 15–17 days in case of 15 days old seedlings have been utilised in vegetative growth to make a total of 30 days and the net period of 'short-days' to induce early flowering is probably only 30–33 days as is the case with older seedlings, *i.e.*, 30 days and over.

It is interesting to note from these studies that a period of 60 days is the very minimum for any variety of paddy to come to flower. The 'Sathi' having 60 days growth period are thus the earliest paddy in nature.

These studies have opened out a new field for hybridization work in paddy by enabling us not only to make paddy varieties widely differing in their normal flowering dates to flower simultaneously, any time in the season, but also to make individual plants of any particular variety to come to flower, one after the other, throughout its growing period—as against hardly for a fortnight at fixed time—and thus get ample material and opportunity for intercrossing it with large number of other varieties and strains with different periods of maturity and flowering dates.

It may incidentally be pointed out that the late variety, which was under study in pots, was also grown in four small beds in one of the paddy fields. When the seedlings were 30 days old, two of them were covered with moveable Tati-covers at 3 p.m. each day. After about 32 days of this treatment, the two experimental beds came to flower. Thus by this light treatment any individual plant or a set of plants in a plot can be made to flower at any suitable earlier date and thus serve a variety of purpose.

It may finally be mentioned that quite a good amount of data have been collected on the growth and yield of the plants subjected to these light treatments, which will form the subject-matter for next paper. But it will not be out of place to mention in a general way that the height of the plants subjected to 'short day' treatment gets increased.

TABLE III

Table showing the effect of shortening the 'day length' at different stages of growth on the flowering dates of different paddy varieties

Age of seedlings before receiving the treatment	Paddy variety	Total number in days for which plants received 'short days' before coming to flower. Figures in brackets show the dates on which the treatment was started in both 3 p.m. and 5 p.m. sets		Date of flowering — Hours for which plants received sunlight		Average period in days of induced early flowering as compared to the control	
		3 p.m. set	5 p.m. set	3 p.m. set	5 p.m. set	3 p.m.set	5 p.m. set
1	2	3	4	5	6	7	8
7 days' old	'Sathi'	(8th June) 53	53	31st July–1st Aug.	31st July–1st Aug.	Flowered with control	Flowered with control
	'Aus' (28–16–21)	54	53	1st–2nd Aug.	31st–2nd Aug.	19	20
	Early (115 BK)	53	55	31st July–1st Aug	2nd–3rd Aug.	69	67
	'Aman' Medium (16 BK)	55	53	2nd–4th Aug.	31st July–2nd Aug.	79	81
	Late (36 BK)	53	55	31st July–2nd Aug.	2nd–4th Aug.	89	87
15 days' old	'Sathi'	(15th June) 47	45	2nd–3rd Aug.	31st July–2nd Aug.	Flowered with control	Flowered with control
	'Aus' (28–16–21)	45	46	31st July–2nd Aug.	1st–2nd Aug.	20	19
	Early	47	45	2nd–4th Aug.	1st–2nd Aug.	67	68
	'Aman' Medium Do.	45	45	31st July–3rd Aug.	31st July–3rd Aug.	81	81
	Late	46	47	1st–3rd Aug.	2nd–3rd Aug.	88	87
30 days' old	'Sathi'	(30th June) 31	30	31st July–1st Aug.	30th–31st July	Flowered with control	Flowered with control
	'Aus' (28–16–21)	29	30	29th July–1st Aug.	30th July–2nd Aug.	22	21
	Early	32	30	1st–4th Aug.	30th July–3rd Aug.	68	69
	'Aman' Medium Do.	29	30	29th July–2nd Aug.	30th July–4th Aug.	73	82
	Late	32	30	1st–4th Aug.	30th July–3rd Aug.	88	90

Age of seedling	Variety	Date of transplanting	No. of days to flower		Date of flowering		No. of days	
45 days' old	'Aus' (28-16-21)	(15th July)	32	30	15th–18th Aug.	17th–20th Aug.	3	5
	Early Do.		32	30	15th–18th Aug.	17th–20th Aug.	52	54
	'Aman' Medium Do.		35	33	18th–21st Aug.	20th–22nd Aug.	61	63
	Late		36	34	19th–22nd Aug.	21st–22nd Aug.	68	70
60 days' old	Early Do.	(30th July)	32	33	2nd–3rd Sept.	1st–3rd Sept.	37	36
	'Aman' Medium Do.		33	32	1st–4th Sept.	2nd–4th Sept.	48	49
	Late		32	33	2nd–5th Sept.	1st–4th Sept.	57	56
75 days' old	Early Do.	(14th Aug.)	31	32	16th–18th Sept.	15th–18th Sept.	23	22
	'Aman' Medium Do.		32	31	15th–17th Sept.	16th–17th S.pt.	34	35
	Late		32	31	15th–17th Sept.	16th–18th S.pt.	42	43
90 days' old	Early Do.	(29th Aug.)	32	32	1st–3rd Oct.	1st–4th Oct.	7	7
	'Aman' Medium Do.		31	32	1st–2nd Oct.	30th Sept.–2nd Oct.	20	19
	Late		33	31	30th Sept.–4th Oct.	2nd–5th Oct.	26	28
105 days' old	Early Do.	(13th Sept.)	23	22	7th–8th Oct.	8th–9th Oct.	Flowered with control	Flowered with control
	'Aman' Medium Do.		30	29	14th–16th Oct.	15th–16th Oct.	5	6
	Late		32	31	16th–20th Oct.	17th–20th Oct.	11	12
120 days' old	Early Do.	(28th Sept.)	..	..	..	..	Flowered with control	Flowered with control
	'Aman' Medium Do.		18	20	20th–21st Oct.	18th–20th Oct.	Flowered with control	Flowered with control
	Late		29	28	28th–30th Oct.	29th–30th Oct.		

Normal flowering date

Variety	Normal flowering date
'Sathi'	31st July–1st Aug.
'Aus' (28-16-21)	20th–22nd Aug.
Early	8th–10th Oct.
'Aman' Medium	20th–22nd Oct.
Late	28th–30th Oct.

Number of tillers, dry weight of straw and yield per plant does not seem to differ very much from that of the control plants.

Lastly, it may be mentioned that other aspects of the problem, such as the effect of 'short day' treatment, given at the seedling stage on the growth and flowering of the transplanted crop and other inter-related questions are under investigation and the results will be presented later.

SUMMARY AND CONCLUSIONS

A. '*Long day*' *treatment.*

1. By increasing the 'day lengths', *i.e.*, by exposing the plants to artificial light during the night, flowering time of paddy can be suitably delayed—the actual shifting of the flowering dates depends on the duration of the 'long day' treatment given.

B. '*Short day*' *treatment.*

1. Any variety of paddy, irrespective of the class or maturity period to which it may belong, can be induced to flower within 60–63 days of germination.

2. With 'short day' treatment, different paddy varieties with different flowering dates can not only be made to flower simultaneously but individual plants of any particular variety can also be induced to flower, one after another throughout the growing period—thus offering ample material and opportunity for intercrossing it with a number of other varieties with different maturity periods.

3. With this treatment, even 'Aus' varieties, whose period of growth between sowing and flowering is fixed and rather short, can be induced to flower earlier.

4. The 'Sathi' variety did not at all respond to the treatment. Its 60 days normal period of growth could not thus be reduced any further.

5. 'Short day' treatment method has been found to be easier to work with than the 'long day' one specially when Petromax is used as a source of light.

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PROF. S. L. GHOSE

OBITUARY

PROF. S. L. GHOSE

(1893-1945)

A STRANGE fate attends the chair of Botany at Government College, Lahore. The late Professor S. R. Kashyap, who enjoyed robust health and had only the previous year returned from a long trek in the Inner Himalayas and Tibet, died suddenly in 1934, of heart failure, at the early age of 52. Dr. S. L. Ghose, who succeeded him, has now passed away at the same age. Although it was known that he had been keeping indifferent health for some years, none expected that the end would come so soon. The news of his premature death has come as a great shock to his numerous friends, colleagues and pupils.

Dr. S. L. Ghose, born on 13th of December 1893, was the youngest son of Mr. N. C. Ghose, who served for many years in the Education Department of the Punjab and the North-West Frontier Province as Headmaster of several high schools. After a brilliant academic career as a student of the Forman Christian College and the Government College in Lahore, S. L. Ghose took the M.Sc. Degree in Botany of the University of Panjab in 1914, and started life as Demonstrator in Botany in the Allahabad University. He served here for one year. Next year he returned to his *alma mater* as Lecturer in Botany, to work under his own teacher, the late Professor Dr. S. R. Kashyap. He went to Cambridge in 1921 and studied under the guidance of Prof. Seward and Dr. Borradaile. He was awarded the Doctorate in Philosophy in 1923. On his return to India after an extensive tour of continental universities, he was offered the Chairmanship of the newly established Department of Biology at the University of Rangoon. In 1928, however, he returned to the Government College, Lahore. On the death of Dr. Kashyap, he was appointed Professor of Botany. He held this post with distinction until his death on March 24, 1945.

Professor Ghose took keen interest in botanical investigations throughout his life. Some of his earlier papers dealt with the morphology of *Selanginella*, conifers and flowering plants, but his later years were devoted entirely to the study of algology. His investigations on the Myxophyceæ of Northern India and Burma are of an outstanding nature, and by his pioneer work he mapped out for fellow botanists in his country a field hitherto practically unexplored. But more than an investigator, he was a teacher. By his genial temperament and sympathetic approach he had endeared himself to all his pupils. He was always ready to help them even at considerable personal inconvenience, but like a true scientific worker, kept an open mind on the subject under discussion and would never try to enforce his own ideas even on his own pupils.

He was elected Vice-President of the Indian Botanical Society for 1931, 1932 and 1938, and was President of the Society for 1941. He presided over the Botany Section of the Indian Science Congress at Patna in 1933. He was one of the Foundation Fellows of the National Institute of Sciences of India.

By his death, Indian algology loses one of its foremost workers and the University of Panjab an inspiring Professor of Botany.

M. S. RANDHAWA.

REVIEW

Root Disease Fungi. By S. D. Garrett, M.A., D.I.C. Annales Cryptogamici et Phytopathologici, Vol. I, 1944. Waltham, Mass., U.S.A.: The Chronica Botanica Co. ; Calcutta : Messrs. Macmillan & Co., Ltd. Pp. 177. $ 4·50.

THIS notable publication, under the new series '' Annales Cryptogamici et Phytopathologici," edited by Dr. Frans Verdoorn, is perhaps the first of its kind written on the epidemiology of soil-borne disease in crop plants, and contains certainly the first exposition of the principles of Root Disease control. Mr. Garrett is a leading authority on this widely dispersed group of root-infecting fungi, having given a new orientation to the study of these pathogens in their natural habitat, *the Soil*. From the ecological point of view of these soil organisms, precious little work had been done until the classical work of Waksman, Reinking and others was published early this century. There is no doubt whatsoever to-day that the soil, in general, represents a complex microflora actively competing for the organic and inorganic food material resulting in the inevitable chain of events like antagonism, specialization in food requirements, etc. To a large extent the original hypothesis of Waksman, *viz.*, "that there are in most soils a basic cosmopolitan fungus flora of *soil inhabitants*, among which were to be found exotic fungi or *soil invaders*" has found support from both temperate and tropical workers engaged in this problem. Mr. Garrett's book admirably summarizes this and other allied problems.

The contents of the book under review are arranged under the following chapter headings : (1) Introduction ; (2) Parasitic specialization in the root-infecting fungi ; (3) Parasitic activity of the root-infecting fungi ; (4) Influence of soil temperature upon parasitic activity ; (5) Influence of soil moisture content, texture, and reaction upon parasitic activity ; (6) Influence of soil organic content and concentration of plant nutrients upon parasitic activity ; (7) Saprophytic activity of the root-infecting fungi ; (8) Dormancy of the root-infecting fungi ; (9), (10) and (11) Control of root disease in field crops : Crop rotation ; Plant sanitation ; Disease control under the growing crop ; (12), 13 and (14) Control of root disease in plantation crops : on virgin areas ; in mature plantations and on re-planted areas ; special problems ; (15) Control of root disease in glasshouse crops. A very exhaustive bibliography terminates the subject-matter of the entire text and is followed by two indices, general and author.

Of special interest to Plant Pathologists are the chapters on "Control of root disease in field and glasshouse crops". Quite a large number of wilts due to fungal attacks are commonly encountered in every-day cultural practices and in the tropics, particularly in seedling beds. Mr. Garrett has focussed sufficient attention in dealing with the subject-matter that forms these chapters. The book on the whole

presents a logical and sequential case into the various fundamental and applied aspects of root-infecting fungi and the author and the publishers deserve congratulation on bringing this volume out. The student of Botany and the Researchers on Soil Fungi in this country will amply benefit by reading this well-written, concise volume which summarises all the latest researches on this important group of soil micro-organisms.

T. S. SADASIVAN.

ERRATA

Volume XXIV, No. 2, page 70, of the paper "Physiological Studies on Some Members of the Family Saprolegniaceæ—III. Nitrogen Requirements" by K. S. Bhargava.

After paragraph 2, *insert*
    " 2. Medium 1 sodium molybdate.... 1 mg. per 1000 c c."

Last paragraph, last line but one, *read*
    " nitrite " in place of " nitrate ".

GLUCOSE SUCROSE RATIO AND RESPIRATORY DRIFTS IN SUGARCANE

By K. N. LAL AND J. S. SRIVASTAVA

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Benares Hindu University

INTRODUCTION

STUDIES in juice characters of cane (Singh, 1940) have shown marked variations in sucrose and glucose percentages with advance in age of the crop. A more or less inverse relationship has been noted between these two juice characters inasmuch as with increase in age, sucrose content of juice, in general, rises while glucose content shows a decline (Singh, 1941 ; Singh and Mathur, 1938). No attempt has so far been made to trace the relation between the respiration rate of successive nodes and glucose sucrose ratio in juice. In the present paper light is thrown on (i) the effect of development of tillers upon sucrose and glucose percentages in juice and upon respiration rate of nodes, and (ii) the possible relationship between the sugar substrate and respiratory intensity in tillers of varying developmental stages.

METHOD AND MATERIAL

The experiments were conducted on Co. 312 grown on the Experimental Farm of the College.   Towards the end of the adolescent stage (10 months) few healthy bushes were sampled. Tillers of various developmental stages were picked up and arranged in accordance with their increasing height, begining from the youngest on one side and the old well mature tillers on the other. A number of such tiller classes, 1–15, were thus grouped. The entire group contained tillers of varying age ranging between 5–6 months (tiller classes 1–5), 6–7 months (tiller classes 6–9), 7–8 months (tiller classes 10–12) and 8–10 months (tiller classes 13–15).   Stripped canes belonging to each of these classes were divided into three equal size pieces and designated as top, middle and bottom canes according to their respective position on the shoot. Green tops were invariably discarded. Each of these portions were

separately crushed and requisite quantity of juice was analysed with respect to sucrose and glucose percentages. Sucrose was estimated by saccharimeter and glucose by Fehling's solution.

Respiration rate of representative nodes (4″ in length) from each of the three pieces (top, bottom and middle) and selected from all the different classes of tillers was determined at 31° C. by continuous current method using standard baryta solution as an absorbent. Respiration of the top-most node in the green non-millable cane was also determined. All records have been expressed in milligrammes of carbon dioxide per 100 gm. fresh weight per hour. The cut end of the nodes were invariably sealed with a wax-vaseline mixture and allowed overnight rest before actual respiration measurements were undertaken.

EXPERIMENTAL RESULTS

A. *Sucrose Glucose Percentages and Respiratory Drifts in Top Portion of Cane*

Respiration rate of topmost node situated in the green non-millable cane varied enormously from tiller to tiller (Table 1). In the

TABLE 1

Respiration from sugarcane sets from various heights on the cane shoot in different tillers of a bush

(Resp. in mgm. carbon dioxide per 100 gm. fresh weight per hour)

Tiller No.	Position of the sets on the tiller				Total
	Top-most	Top	Middle	Bottom	
1	170·23	118·30	95·20	66·44	450·19
2	148·87	147·34	82·20	57·47	435·97
3	143·29	117·07	69·27	75·65	405·18
4	126·34	86·21	85·18	62·25	359·45
5	155·04	88·80	60·10	42·01	345·98
6	38·19	35·55	29·23	24·49	127·46
7	28·30	14·66	11·77	11·80	67·53
8	20·41	12·83	7·18	8·78	49·46
9	17·36	16·02	12·05	10·90	56·28
10	22·19	8·86	5·38	3·55	39·04
11	15·32	13·12	8·08	8·78	45·31
12	13·28	10·83	4·05	4·77	32·95
13	27·30	10·76	10·73	10·11	38·90
14	22·78	21·59	10·35	14·90	69·09
15	29·06	11·67	11·01	11·24	61·01

youngest tiller it was highest, gradually decreased with advance in age of the tiller and finally attained a very low level in the oldest cane of the bush. All the younger tillers (5–6 months old) showed a relatively high rate of carbon dioxide output. There was, in general, a continuous slow decline in respiratory activity with increase in age, which attained a very low level in the twelfth tillers (8 months old) of the

bush. Slight rise in activity was again noted in the 8–10 months old canes (Tiller number 13–15), but the increases observed seldom attained the values recorded for the tillers of younger age.

Respiration rate of another node situated at a slightly lower level than that of the above also showed a more or less similar high rate of respiration in younger 5–6 months old tillers and a gradual fall with advance in age till a more or less level course was attained in 8–10 months old canes (Table I, Fig. 1). In almost all tillers the topmost node exhibited a higher rate of respiration than that situated slightly below it. The differences in respiration rate were more discernible in the younger tillers than in the old. High rate of respiration of the top and topmost nodes of 5–6 months old tillers, was apparently due to the high concentration of reducing sugars in these regions, as also the greater protoplasmic activities in the younger regions of tiller number 2–5. The younger the age of the tiller, the higher was the concentration of reducing sugars and in consequence the greater were the rates of respiration.

Sucrose content of the top canes on the contrary, exhibited the reverse course. In the youngest tiller the percentage was low ; it gradually rose to a high value with increase in age of the cane. Tiller number 5–8 showed a level course of sucrose. Further increase in age resulted in still higher values of sucrose (Table II, Fig. 1). Marked

TABLE II

Sucrose percentage of the sets from various heights on the stem of different tillers in a bush

Tiller No.	Position of the sets on the tiller			Total
	Top	Middle	Bottom	
1	8·30	12·20	12·30	32·90
2	10·70	12·80	14·50	39·00
3	13·20	15·05	14·50	42·75
4	14·50	· 15·70	16·05	46·25
5	13·10	15·10	15·80	44·00
6	13·60	16·40	15·80	45·80
7	13·05	13·10	11·45	37·60
8	13·60	17·70	17·40	48·70
9	17·30	17·30	17·20	51·80
10	16·30	16·90	16·70	49·90
11	15·40	18·20	18·10	51·70
12	·16·60	18·20	16·40	50·20
13	14·70	17·30	13·10	45·10
14	19·20	18·00	17·00	54·20
15	17·95	17·25	16·35	51·55

fluctuations were, however, noted in the sucrose content of the entire group of old tillers. These did not coincide with the fall or rise in respiration rate always, although occasionally some such relation was exhibited,

The glucose content, on the other hand, exhibited a course which was more or less reverse to that of the sucrose percentage in the old tillers and a slightly parallel course in the younger 5–6 months old tillers.
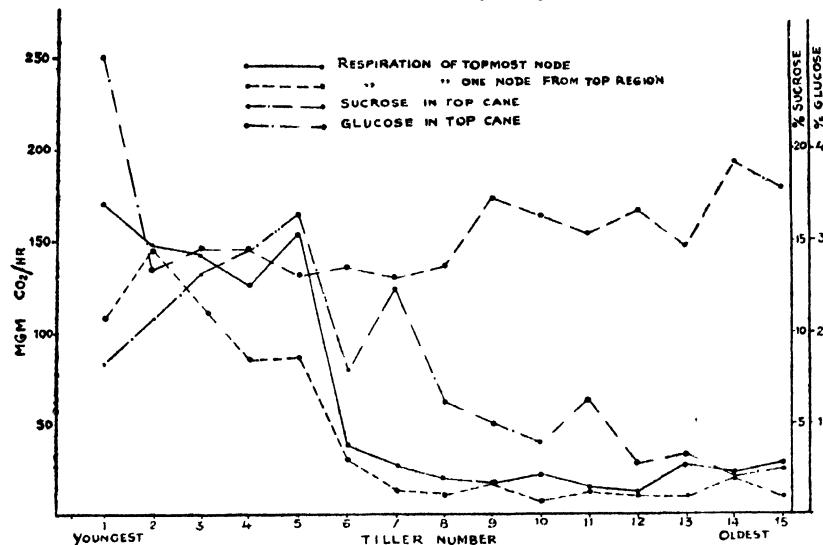
Fig. 1. Respiration, and sucrose and glucose percentages in tillers of various developmental stages. Relative age of tillers: Nos. 1–5 (5–6 months), Nos. 6–9 (6–7 months), Nos. 10–12 (7–8 months), and Nos. 13–15 (8–10 months).

The fall in glucose concentration particularly in the old canes invariably coincided with the rise in the sucrose value and *vice versa* (Table III,

TABLE III

Glucose percentage of the sets from various heights on the stem of different tillers in a bush

Tiller No.	Position of the sets on the tiller			Total
	Top	Middle	Bottom	
1	5·00	4·10	2·20	11·30
2	2·70	2·50	2·50	7·70
3	2·90	2·50	2·00	7·40
4	2·90	2·50	2·50	7·90
5	3·30	2·50	1·80	7·60
6	1·60	1·10	1·40	4·10
7	2·50	2·00	2·50	7·00
8	1·25	1·25	1·00	3·50
9	1·00	0·71	1·00	2·71
10	0·82	1·00	0·71	2·53
11	1·25	1·00	0·71	2·96
12	0·55	0·50	0·15	1·20
13	0·66	0·30	0·50	1·46
14	0·42	0·55	0·91	1·88
15	0·50	0·54	0·71	1·75

and Fig. 1). High glucose and low concentration of sucrose were usually associated with high respiration, and low glucose and high sucrose with low respiration rates.

B. *Sucrose Glucose Percentages and Respiratory Drifts
in Middle Portion of Cane*

In the middle portion of different canes the course of respiration of a single node again showed a fall with advance in age of tillers. The fall was sharp in the younger 5–7 months' old tillers, but attained more or less a level course throughout the 7–10 months' old canes (Table 1, Fig. 2). Sucrose content, on the contrary, was increasing
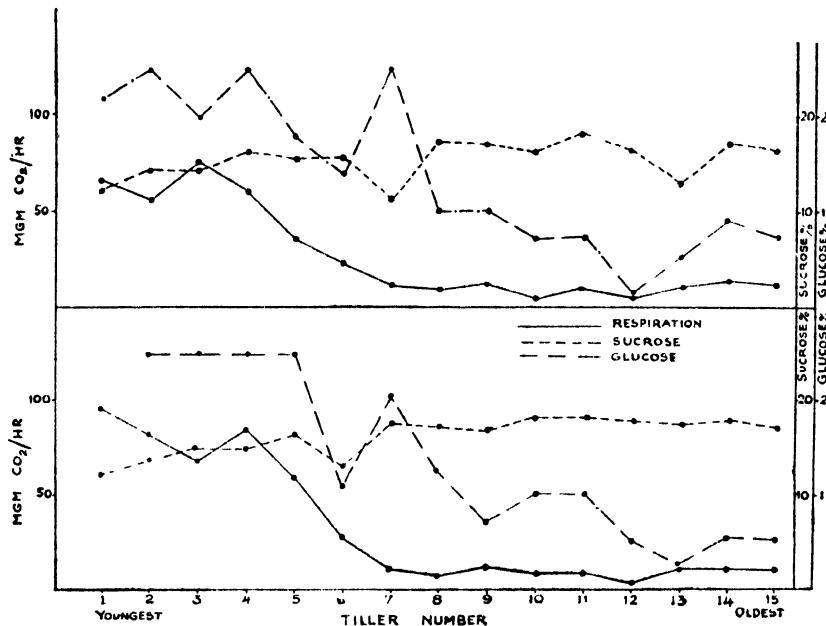
Fig. 2. Respiration, and sucrose and glucose percentages in middle and bottom portion of cane from tillers of different developmental stages—middle (below), bottom (above). Relative age of tillers : Nos. 1–5 (5–6 months), 6–9 (6–7 months), 10–12 (7–8 months), and 13–15 (8–10 months).

with advance in age, attained a high but level phase in the older (8–10 months) tillers and showed only a negative relation with respiration. The concentration of reducing sugars in the younger canes was more or less constant, but fell down as the age advanced. The fall in glucose concentration was more marked in spite of similar level of sucrose in older tillers.   Glucose again showed some parallelism with the course of respiration while sucrose indicated only, if at all, a reverse relationship ; this was, how ever, not as conspicuous as in the top portion of the cane. Here again· high rate of respiration was due to the relatively high concentration of reducing sugars ; the younger the respiring node, the more was the glucose concentration and the higher were the respiration rates (Tables I and III),

C. *Sucrose Glucose Percentages and Respiratory Drifts in Bottom Portion of Cane*

The course of respiration of a node collected from the basal portion of the cane also followed the same general sequence of decline with advance in age of the respective cane. The sucrose content showed a rising tendency, attained high percentage in 8–10 months' old canes and was maximum in the eleventh tiller of the bush (Fig. 2). Subsequent advance in age of the tillers lowered the percentage of sucrose slightly. Glucose showed a general decline with increasing age and reached lowest concentration in the twelfth tiller. Further advance in age caused a rise in glucose concentration ; this was evident in 10 months' old canes of the bush. No relationship was observed between the rise or fall in glucose and sucrose values in the different canes.

D. *Average Respiration Rate and Glucose/Sucrose Ratio*

The average respiration rate of a cane and the average glucose and sucrose percentages are shown in Table IV and Fig. 3. While

TABLE IV

Average respiration and juice characters of various tillers

Tiller No	Respiration	Sucrose	Glucose	Sucrose / Glucose	Glucose / Sucrose
1	112·54	10·96	3·77	2·91	3·44
2	108·99	13·00	2·56	5·08	1·97
3	101·29	● 14·25	2·46	5·79	1·72
4	89·86	15·08	2·63	5·73	1·74
5	86·49	14·66	2·53	5·79	1·72
6	31·86	15·26	1·36	11·22	0·08
7	16·63	12·53	2·33	5·73	1·86
8	12·36	16·23	1·16	14·00	0·07
9	14·07	17·26	0·90	19·18	0·52
10	9·76	16·63	0·84	19·79	0·05
11	11·33	17·23	0·99	17·40	0·05
12	8·24	16·73	0·40	40·18	0·02
13	9·72	15·03	0·49	30·68	0·03
14	17·27	18·07	0·63	28·69	0·03
15	15·25	17·18	0·58	29·60	0·03

the average rate of respiration exhibited a gradual decline with advance in age of the tiller, the average sucrose content showed an increase. Glucose concentration varied more or less in the same direction as respiration rate though the parallelism was not evidenced sharply at all stages of the development of the tiller. The sucrose/glucose ratio exhibited a low value in the younger,5–7 months' old canes, and a gradual increasing value in the older ones. Glucose/sucrose ratio, on the contrary, indicated high values in the younger tillers and a very low value in 8–10 months' old canes. Respiration showed strictly parallel course with glucose/sucrose ratio except in the case of the seventh tiller (Fig. 3). The younger the age, the more was the rate of

respiration ; this high rate was invariably associated with high con-
centration of glucose, low sucrose and high glucose/sucrose ratio.
Approximately sevenfold increase in respiration rate in the youngest
cane was in confirmity with 4–7 times rise in glucose percentage and
6–10 times increase in glucose/sucrose ratio (Table IV).

Variations in respiration rate and sucrose and glucose percentages
in tillers of various stages of development point out the importance
of respirable substrate on the output of carbon dioxide. Glucose
concentration appears to have a controlling influence upon cane res-
piration, and the ratio that it bears to the sucrose percentage (glucose/
sucrose ratio) shows more rigid a relationship to the intensity of
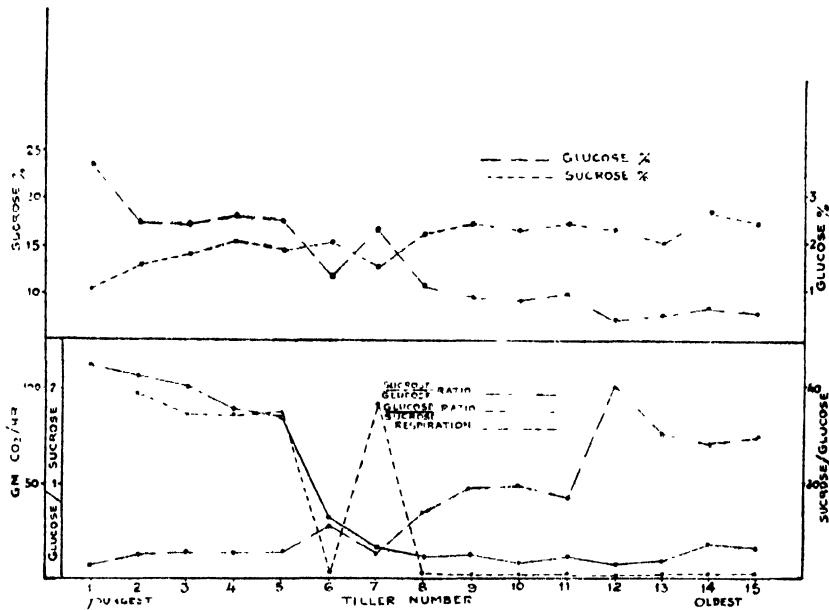
Fig. 3. Average glucose and sucrose percentages in cane juice and sucrose/
glucose and glucose/sucrose ratios in relation to respiration rate of canes. Age of
tillers : Nos. 1–5 (5–6 months), 6–9 (6–7 months), 10–12 (7–8 months), and 13–15
(8–10 months).

respiration. In younger tillers, intensity of metabolism and rapidity
of growth, demand quick supply of easily assimilable sugars and this
is amply secured by the high proportion of glucose in comparison
to sucrose in young tillers. In old canes, where tissue development
has proceeded beyond a certain limit, the demand of the less active
tissues on the readily respirable substratum falls down considerably
and this explains why there is a preponderance of more stable sucrose
over the reducing sugars. The younger tillers are in a way more
physiologically active than the older canes in a single bush. In any
comparative study of respiration rates of varieties, or for the matter
of that, of other treatments, therefore, care needs to be taken to

extend respiration measurements to as many tillers of the bush as practicable so as to get a representative picture of the nature of response.

SUMMARY

The paper deals with the respiratory drifts in canes of various stages of development selected from a field crop of sugarcane var. Co. 312. Height of the shoot was taken as the criterion for the classification of the various groups of tillers. Fifteen groups were sampled and arranged in order of increasing height ; these included canes 5–6 months' old (tiller number 1–5), 6–7 months (tiller number 6–9), 7–8 months' (tiller number 10–12), and 8–10 months' old cane (tiller number 13–15). The millable portion of these stripped canes only were selected and equally divided into three parts—top, middle and bottom.

Respiration rate of a single node in sets four inches long and collected from (i) extreme apex in the green non-millable cane, (ii) top region, (iii) middle portion and (iv) bottom piece of each of the above tillers was measured. Sucrose and glucose concentrations in extracted juice from top, middle and bottom pieces were separately estimated.

Irrespective of the portion from which a set was selected for measurement of respiration rate, the intensity of carbon-dioxide output declined with each successive increase in age of tillers. Younger tillers were physiologically more active than the old and mature canes. In each class of tillers again the respiratory activity was highest at the top and decreased as one proceeded downwards.

Variations in respiration rate were found to be related to fluctuations in glucose more than sucrose, particularly in tillers of the older group. In almost all tillers, glucose/sucrose ratio appeared to determine the intensity of respiration of cane sets. Physiological significance of such changes has been discussed.

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SOME FOSSIL LEAVES OF THE ULMACEÆ FROM THE KAREWA DEPOSITS OF KASHMIR

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INTRODUCTION

THE material described in this paper is mainly derived from collections made at Laredura partly by Dr. R. R. Stewart in 1935, and partly by the author in 1939. There are also two leaf fragments gathered by Dr. H. de Terra from Gogajipathri and Liddarmarg ; and one leaf impression collected by Stewart in 1936 from Ningal Nullah. The plant-bearing outcrops lie in the Baramulla-Gulmarg region and are assigned the Lower Pleistocene age (De Terra and Paterson, 1939).

List of the Species

Species	Localities	References to Figures
Ulmus Wallichiana	Laredura, 6,000 ft.	Figs. 1, 2
Ulmus campestris ..	Gogajipathri, 8,800 ft. and Laredura, 6,000 ft.	Figs. 3, 4, and 8
Ulmus laevigata ..	Laredura, 6,000 ft. and Ningal Nullah, 9,000 ft.	Figs. 5, 6, 7
Ulmus sp. ..	Liddarmarg, 10,600 ft.	Figs. 9, 10

DESCRIPTION

Order : Urticales.

Family : Ulmaceæ.

The family is represented in the Karewa flora by a number of leaf impressions, all referable to the modern genus *Ulmus*. The genus *Ulmus* probably had a fairly wide distribution in the Valley during the Lower Pleistocene, which is evidenced by the discovery of one or the other species from four different localities including Laredura, Ningal Nullah, Gogajipathri and Liddarmarg. The genus includes three species

3

two of which, *viz., Ulmus Wallichiana* Planch. and *U. lævigata* Royle are discovered together from the beds at Laredura. The third species, *Ulmus campestris* Linn., has been found from Gogajipathri. Fruits of *Ulmus* spp. are small and are provided with circular wings, like samaras of *Litchi.* They are likely to be represented in the fossil beds as they are capable of flight over long distances like the samaras of *Acer* and *Fraxinus,* which have also been discovered in the beds that have yielded leaves of these genera, but a very close study of the present collections has failed to bring to light any fruits of this genus.

<div align="center">GENUS Ulmus LINN.</div>

The genus includes three well determined species, the modern representatives of which are still growing in the Kashmir Valley and the nearby regions. One more leaf fragment, which could not be determined specifically, is also described.

<div align="center">KEY TO THE SPECIES</div>

I. *Leaves large—*

    (*a*) Base very oblique ; laterals 15–17 pairs, running straight and parallel   ..   ..   *U. Wallichiana*

    (*b*) Base not so oblique, leaves comparatively smaller ; laterals 12–13 pairs, curved and not strictly parallel.   *U. campestris*

II. *Leaves much smaller, base rounded or cordate—*

    Laterals about 10 pairs running straight and parallel............
<div align="right">U. lævigata</div>

<div align="center">Ulmus Wallichiana Planch.</div>

<div align="center">(Pl. XI, Figs. 1–2)</div>

Plate XI, Figs. 1–2 are two natural size photographs of a leaf and its counterpart ; a small piece from the base on left-hand side in one specimen (Pl. XI, Fig. 1) got chipped off while splitting the clay to expose the two counterparts. The leaves are elliptic oblong in outline and measure 3·3 inches long by 1·2 inches in the broadest part, which is midway between the base and apex. The lamina, which is uniformly broad for most of its length, narrows down into a wedge-shaped base, and ends towards its upper part into a slightly curved acute apex. Margins are not well preserved but their dentate nature is clearly seen towards the apical part in Fig. 1. A small curved petiole almost complete in one leaf and slightly broken in its counterpart is also preserved.

The venation is strict-pinnate and reticulate. A stout midrib arises from the oblique base and runs in the lamina gradually thinning out towards the apical part. It follows a curved course dividing the lamina into unequal halves. 16–17 secondaries, which are about half as thick as the midrib, diverge from the latter, on either side, at acute angles. The origin of the laterals, especially in the lamina above the middle, is mostly alternate, but some laterals towards the lower portion tend to become sub-opposite ; they run straight to the

margins, parallel to one another, and each ends in a marginal tooth. The laterals, as well as the midrib, have left shallow grooves in the impression (Fig. 1) and stand out in the form of ridges in its counterpart. A comparison with modern leaves of this species shows that the former is an impression from the lower surface of the leaf whereas its counterpart represents the upper surface. The tertiary ribs are not well developed. The finer reticulation consists of a well preserved net-work of small, oval, or circular meshes, which are seen at some places in Fig. 2.

The fossils are identical with modern leaves of *Ulmus Wallichiana* Planch., a common western Himalayan elm.

Number of specimens.—Ten only.

Occurrence.—Laredura, at 6,000 ft. in the Pir Panjal Range, Kashmir.

Collection.—R. R. Stewart, 1935.

Registered numbers of figured specimens.—Pl. XI, Fig. 1=L 620 ; Pl. XI, Fig. 2=L 621.

<center>*Ulmus lævigata* Royle</center>

<center>(Pl. XI, Figs. 5–6 ; Pl. XII, Fig. 7)</center>

This species is based on a number of leaves, two of which are illustrated in natural size in Pl. XI, Figs. 5, 6. The figured leaves are ovate in outline measuring 1·15 inches long by ·35 inch in the broadest part, which lies midway between base and apex. One leaf (Pl. XI, Fig. 5) is slightly broken on one side along the margin. This specimen was coated in field with a thick layer of rubber solution, which has completely obscured the finer reticulations. The margins are mostly broken in the impressions but their serrate nature in one leaf (Pl. XI, Fig. 6) is clearly seen at some places in Fig. 7, which represents a part of the leaf enlarged to five diameters. Base is rounded, or slightly sub-cordate. Apex is acute.

The venation is strict-pinnate and reticulate. A fairly strong midrib runs in the lamina gradually thinning out towards the apical part. It usually runs straight but in Fig. 5 it follows a slightly curved course in a part of the lamina. 10–13 pairs of laterals, which are almost as thick as the midrib, diverge from it at acute angles ; they run straight in the lamina parallel to one another ; their manner of origin is mostly opposite, but some of them arise rarely in an alternate manner. The laterals usually end in the marginal teeth, which are mostly sharp pointed. The tertiary and finer reticulations are greatly obscured in one leaf and are badly preserved in the other, but the finer reticulation is seen at some places in the enlarged photograph.

The fossils are identical with *Ulmus lævigata* Royle, the small leaved elm of the Punjab Himalayas.

Number of specimens.—Eight only.

Occurrence.—Laredura, at 6,000 ft. and Ningal Nullah, at 9,000 ft. Pir Panjal Range, Kashmir.

Collection.—R. R. Stewart, 1935.

Registered numbers of figured specimens.—Pl. XI, Fig. 6=N 161
Pl. XI, Fig. 5=N 186.

Ulmus campestris Linn.

(Pl. XI, Figs. 3, 4 and Pl. XII, Fig. 8)

Two leaf fragments on which this species is based are illustrated
in Fig. 3 on Plate XI and Fig. 8 on Plate XII ; one leaf is slightly
broken at apex and along one side and the other fragment is broken
at the base and both margins. The leaf lamina, which probably had
an elliptic-oblong shape, narrows down to a slightly cuneate and oblique
base. The narrowed nature of the lamina towards the upper part of
the leaf is suggestive of its having an acute apex. The fragments vary
only very slightly in size and the larger measures 2·5 inches long by
1·4 inches in the broadest part, which lies midway between base and
apex. The margins seem to be biserrate.

The venation is strict-pinnate and reticulate. A fairly stout midrib
runs in the lamina from the base gradually thinning out towards the
apical part, and seems to divide the lamina into slightly unequal halves.
10–11 Secondaries, which are hardly half as thick as the midrib,
diverge from the latter on either side at different angles. The lower
pairs of laterals diverge at open angles, whereas the upper few pairs
arise at acuter angles. The origin of the laterals is opposite as well
as alternate ; they tend to run parallel to one another in the lamina,
and end in the marginal teeth. The tertiary ribs are generally not well
preserved but they are seen faintly in one specimen. Plate XI, Fig. 4,
which is a photograph of a part of the leaf enlarged to about five
diameters, shows a nicely preserved network of fine rectangular or
pentangular meshes of the finer reticulation.

The fossils on account of their shape, size, number of laterals,
etc., are identified with living leaves of *Ulmus campestris* Linn. to which
they are identical in all respects.

Number of specimens.—Two only.

Occurrence.—Gogajipathri, at 8,800 ft., and Laredura, at 6,000 ft.,
in the Pir Panjal Range, Kashmir.

Collections.—H. de Terra, 1932 and G. S. Puri, 1940.

Registered numbers of figured specimens.—Pl. XI, Fig. 3=Loc. 2
G 6 ; Pl. XII, Fig. 8=L 823/2.

The Fourth Species

(Pl. XII, Figs. 9 and 10)

In addition to the three above described species there is one more
leaf fragment, which resembles *Ulmus Wallichiana* Planch. in some
features and in others it compares with *Ulmus campestris* Linn.; there
are, however, a few additional features, which are entirely new to
either of the two species. Pl. XII, Fig. 9 is a natural size photograph
of the fragment, which is badly broken on apex, base and the two
margins ; its venation is very clearly preserved and characteristic of the

genus.   The laterals are seen in the photograph in the form of well marked ridges, which show that the fossil is probably an impression from the upper surface of the leaf.

The features of this leaf are compared to the modern leaves of the two fossil species in a tabular form below :—

Characters	*Ulmus* sp. (Pl. XII, Figs. 9–10)	*U. Wallichiana* Planch. (Pl. XI, Figs. 1, 2)	*U. campestris* Linn. (Pl. XI, Figs,3, 4 and Pl. XII, Fig. 8)
1. Shape of the lamina	Not known exactly but resembles more with *U. campestris* Linn. than the other species.	Elliptic oblong	Probably elliptic
2. Numer of the laterals	About 8 pairs in the fragment, which is almost complete and a complete leaf might have, at the most, three or four pairs more.	16–17 pairs.	10–11 pairs.
3. Nature of the laterals (i) angle of origin (ii) manner of divergence	Arise at acute angles and resemble *U. Wallichiana* in this feature; they are curved and do not run parallel.	Arise at acute angles ; they are straight and run parallel.	Arise at comparatively less acute angles, and the lower pairs arise at open angles ; they are curved and do not run exactly parallel.
(iii) Branches of laterals	Some laterals give off branches near the margin. Secondaries arise at unequal distances.	Laterals do not give off branches. Arise at equal distances.	One or two laterals give off branches near the margins. Do uot arise at equal distances.
Tertiary ribs	Very well marked out and conspicuous, form cross-ties, or sometimes large, rectangular meshes ; they are about half as thick as the laterals.	Not seen in a large part of the leaf ; in modern leaves they are thinner than the laterals and are not at all well marked out.	Not seen in the fossil, but in modern leaves they are as conspicuous as in *U.Wallichiana*.
5. Finer reticuculations	Meshes smaller, like *Ulmus Wallichiana*.	Meshes small.	Meshes comparatively larger.

From the above comparison it seems that this leaf cannot be placed in either of the two species ; therefore, it is described separately as *Ulmus* sp., which is different from all modern species of *Ulmus* represented in the flora of the Himalayas.

Number of specimen.—One.

Occurrence.—Liddarmarg at   10,600 ft., in the Pir Panjal Range, Kashmir,

Collector.—H. de Terra, 1932.

Registered number of the figured specimen.— Loc. 3 L 100.

Modern Distribution of the Ulmaceæ

The family Ulmaceæ, which includes 130 species distributed in 13 genera of modern plants is, at the present time, well represented in the tropical and extra-tropical regions of the world. The most northerly point of its occurrence in the New World is 43° 30′, whereas it reaches 58° N. and 66° 59′ N. in Asia and Europe respectively. The chief genera are *Ulmus*, *Celtis* and *Trema*.

The genus *Ulmus*, with 18 species, is distributed mainly in the North temperate zone, and occurs also in the mountainous regions of tropical Asia. Two species, namely, *Ulmus montana* and *U. campestris* are typically British ; the latter is also found in north-western Europe and western Asia, and occurs in India probably as a cultivated tree at Ghoragali in the Murree Hills.

In India we have only five species—of which two (*Ulmus Wallichiana* and *U. lævigata*) are common elms of the Western Himalaya ; one species (*U. lancifolia*) is the Eastern Himalayan elm found in Sikkim, Bhutan, Assam, Chittagong and Burma ; *U. parvifolia* an evergreen shrub, occurs wild, according to Brandis, in Nubra, northern Kashmir ; the fifth Indian species is *U. campestris*, the common elm of Europe, which occurs in Baluchistan and the Kurram Valley.

Modern Distribution of the Fossil Species

The Karewa flora includes four species of *Ulmus*, three of which are definitely determined but one leaf-impression could not be specifically determined on account of its fragmentary nature.

Ulmus Wallichiana—the common West Himalayan elm—is a large deciduous tree, which occurs from the Indus to Nepal at 3,500 to 10,000 ft. (not in gregarious patches), among the coniferous as well as broad-leaved forests. It usually grows in moist ravines but it is not uncommon on dry slopes, where it is mostly stunted. It is able to colonise landslips, banks of ravines and other waste places with amazing rapidity.

In the " mixed coniferous forests of Grahan Nal," Parbatti Valley, Punjab, *Ulmus Wallichiana* occurs at 7,000 ft. to 9,000 ft. in association with *Cedrus Deodara*, *Abies Pindrow*, *Picea morinda*, *Juglans regia*, *Corylus colurna*, *Celtis australis*, *Acer* spp., etc. (Champion, 1936, p. 243).

It also occurs in the "eastern oak—fir forest of Garhwal Himalaya" with *Abies Pindrow*, *Picea morinda*, *Quercus semecarpifolia*, *Q. dilatata*, *Aesculus indica*, *Acer Cæsium*, *Corylus colurna*, *Rubus niveus*, *Rosa macrophylla*, *Skimmia Laureola*, *Syringa Emodi*, *Viburnum* spp., *Hedera Helix*, etc., etc. (Champion, *loc. cit.*, p. 245).

The "moist temperate deciduous forests of Dwali", Western Almora division, Kumaon, are composed of *Ulmus Wallichiana*, *Aesculus*

indica, Acer Cæsium, A. pictum, Carpinus viminea, Betula alnoides, Juglans regia, Fraxinus micrantha, Quercus semecarpifolia, Corylus colurna, Cornus macrophylla, Rhus punjabensis, Taxus baccata, Berberis sp., *Prunus undulata,* etc., etc. (Champion, *loc. cit.,* pp. 257–58). At Saran, in the Kagan Division of Hazara, *Ulmus Wallichiana* is associated with *Juglans regia, Acer Cæsium, Aesculus indica, Prunus padus, Pinus excelsa, Viburnum fœtens.* The same forests of the Sutlej Valley in the Punjab comprise of *Ulmus* spp., *Acer Cæsium, A. pictum, A. villosum, Aesculus indica, Betula alnoides, Carpinus* sp., *Celtis australis, Fraxinus micrantha, Juglans regia, Pyrus lanata, Prunus cornuta, Abies Pindrow, Cornus* sp., *Corylus colurna, Rhododendron arboreum, Rhus* sp., *Viburnum* spp., etc., etc. (Champion, *loc. cit.,* p. 258).

In Kashmir the species occurs in the Valley proper, Gurez, the Jhelum Valley, Keran, Kishtwar, Marwa Dacchan, Muzaffarabad, Ramban, and the Sindh Valley.

The second Karewa species—*Ulmus lævigata (U. villosa)*—is the small-leaved elm of the Punjab Himalaya, which occurs at lower elevations than the former species. In the valleys of Punjab rivers it ascends to as high as 10,500 ft., but usually it is not commonly found above 7,000 ft. It is fairly common in Kashmir occurring in the Valley proper, and Kamraj, Kishtwar, Marwa Dacchan, Muzaffarabad, Ramban and the Sindh Valley. Eastwards it occurs at Munali, Kulu and extends as far as the Pabar Valley. It is also found in Hazara and Murree Hills, the adjoining regions of Kashmir.

The third Karewa species—*U. campestris*—which is the common elm of Europe, occurs in India in the Kurram Valley at 7,000–9,000 ft. and in Baluchistan. It is cultivated in Kashmir and also in Ghoragali in the Murree Hills.

SUMMARY

1. The family Ulmaceæ is represented in the Karewa flora (Pleistocene) of the Kashmir Valley by four species belonging to the single genus *Ulmus;* three of these, namely, *U. Wallichiana, U. lævigata,* and *U. campestris* are based on leaf impressions collected by De Terra, Stewart and the author from Laredura, Ningal Nullah and Gogajipathri ; the fourth is an incompletely determined species, based on a leaf fragment collected at Liddarmarg by De Terra in 1932.

2. At the present time the family, with its 13 genera and 130 species of modern plants, is distributed in the tropical and extra-tropical parts of the globe, whereas the genus *Ulmus* is mainly represented in the north temperate zone ; however, some species are also found in the mountainous regions of tropical Asia.

3. Of the three species described in this paper, two (*U. Wallichiana* and *U. lævigata*) are found in the Western Himalaya, while the third (*U. campestris*), an European elm, occurs in the Kurram Valley, and is also cultivated in Kashmir and Ghoragali in the Murree Hills. The former two species are also common in Kashmir occurring in the Valley and other parts of the Jammu and the Kashmir Territories.

LITERATURE CITED.

Champion, H. G. (1936) ..   " A preliminary survey of the Forest types of India and Burma," *Ind. For. Rec.*, New Series, **1**, *Silviculture*, pp. 243, 245 and 248.

De Terra, H. and T. T. Paterson (1939)   " Studies on the Ice Age in India and associated human *cultures*," Carnegie Institution, Washington.

EXPLANATION OF PLATES

All figures in Plates XI-XII are from untouched negatives. Figured specimens are preserved in the Botany Museum, University of Lucknow.

PLATE XI

Ulmus Wallichiana Planch.

Fig. 1. Leaf fragment (impression of the lower surface). R. R. Stewart collection, L 620 Laredura, 6,000 ft. Nat. size.

Fig. 2. Counterpart of Fig. 1, L. 621. Nat. size.

Ulmus campestris Linn.

Fig. 3. Fossil leaf impression. H. de Terra collection. Loc. 2 G. 6 Gogajipathri, 8,800 ft. Nat. size.

Fig. 4. A part of the leaf (marked × × in Fig. 3) enlarged to show meshes of the tertiary and finer reticulations. × *Ca.* 5.

Ulmus lævigata Royle.

Fig. 5. Fossil leaf. R. R. Stewart collection. L 186 Laredura, 6,600 ft. Nat. size.

Fig. 6. Leaf impression. R. R. Stewart collection. N 161 Ningal Nullah. Nat size.

Fig. 7. A part of the leaf (marked × × in Fig. 6). Enlarged to show serrate margin ; meshes of tertiary and finer reticulation. × *Ca.* 5.

PLATE XII

Ulmus campestris Linn.

Fig. 8. Leaf impression. G. S. Puri collection. L 823/2 Laredura, 6,000 ft. Nat. size.

Ulmus sp.

Fig. 9. Leaf impression. H. de Terra collection. Loc. 3 L 100 Liddarmarg, 10,600 ft. Nat. size.

Fig. 10. A part of the leaf (marked with × × in Fig. 9) enlarged to show tertiaries and finer reticulations. × *Ca.* 5.

SOME FOSSIL LEAVES OF THE ULMACEÆ FROM THE
KAREWA DEPOSITS OF KASHMIR
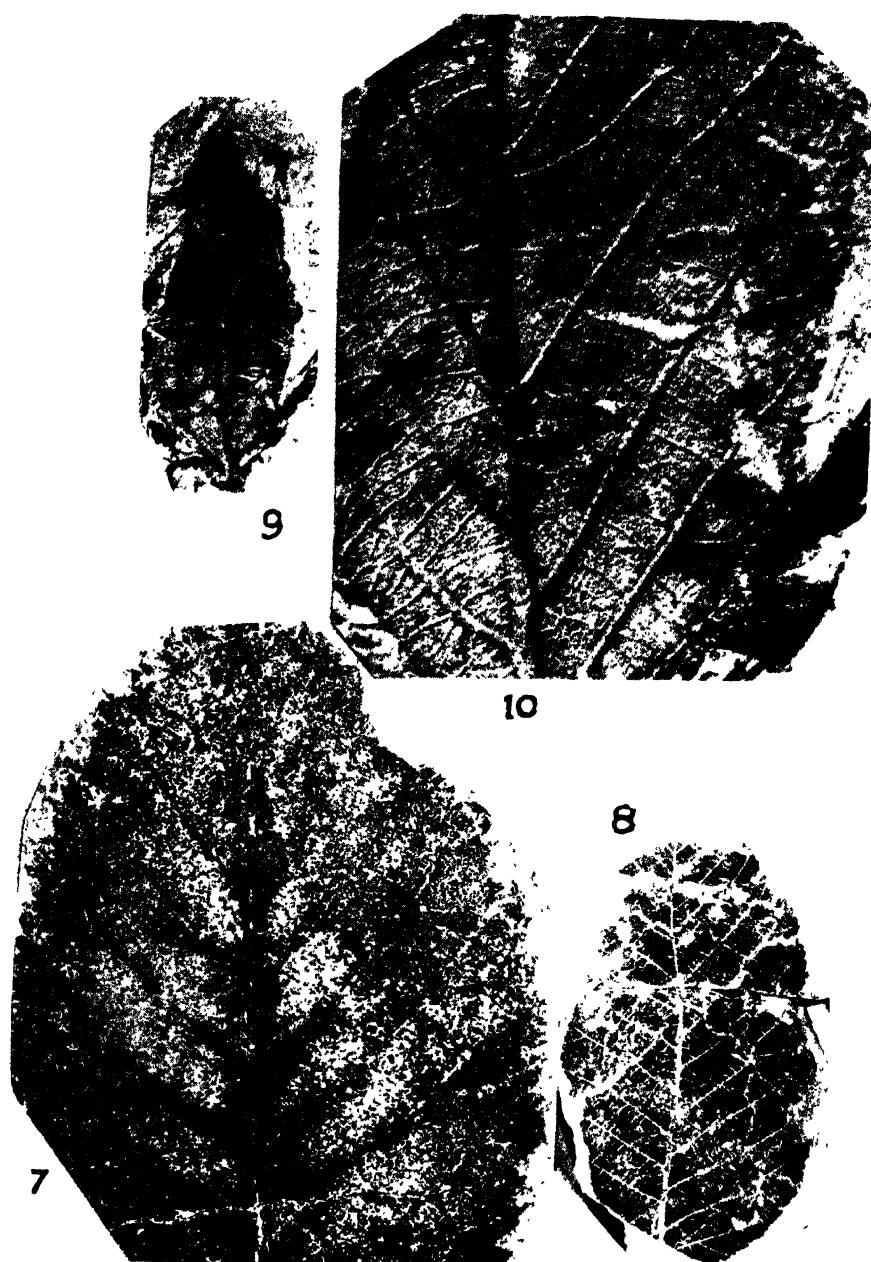
G. S. PURI

*SOME FOSSIL LEAVES OF THE ULMACEÆ FROM THE
KAREVA DEPOSITS OF KASHMIR*

THE ORIGIN, DEVELOPMENT AND MORPHOLOGY OF THE OCHREA IN *POLYGONUM ORIENTALE* L.

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INTRODUCTION

OCHREA (or Ocrea) is a distinctive character of the family *Polygonaceæ*, but its morphology is not exactly clear. Asa Gray (1879) describes it as a pair of stipules united *inter se* in a sheath. Goebel (1905) considers ochrea as "axillary stipules", while Jackson (1916) defines it as "a tubular stipule, or a pair of opposite stipules so combined". Sinnott and Bailey (1914) explained ochrea as " a row of adjacent stipules each opposite one of the numerous leaf-trace bundles, which have become fused together". In view of these differences of opinion it was considered worth while to investigate the morphology of the ochrea in *Polygonum orientale* L. from the organisational and developmental points of view.

Material consisting of growing apices from adult plants was fixed in FAA either directly or after pre-treatment for half an hour in Carnoy's fluid. Sections were cut 8μ–10μ thick, stained with Safranin-Fast Green, Safranin-Hæmatoxylin (Heidenhain's) combinations, or with Fast Green alone.

EXTERNAL MORPHOLOGY

The leaves of *Polygonum orientale* are arranged in two-fifth phyllotaxy. They are 10–22·5 cm. long, 5–12·5 cm. broad, petiolate, ovate-cordate, entire, acute and softly hairy on both sides. The ochrea is 1·75–3 cm. long and from its point of insertion completely envelops the next higher internode of the growing shoot. It consists of two portions, the lower sheathing base and the free tubular upper region (Fig. 1, *sh.* and *fr.*). The sheathing base is about 0·9–1·5 cm. long, is markedly striated, green, hairy on the outside and completely encircles the stem. The free tubular upper region of the ochrea is faintly nerved, grey with hairs and ends in a spreading recurved margin with a distinct notch on the side facing the leaf. The sheathing base persists as a brown scaly membrane after the leaf-fall, while the tubular upper region withers away. The terminal bud forms a small cone covered by the ochreas of several successive leaves. Here the lamina of any leaf is not protected by its own ochrea, but by that of the next

4

older one. The axillary buds are protected by the sheathing portion
of the leaf-base until they grow beyond it.
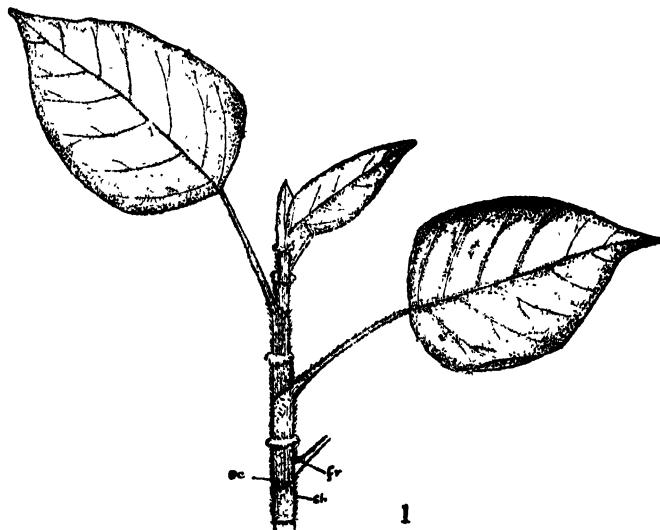
Fig. 1. *Polygonum orientale.*—A part of the shoot showing the ochrea (*oc*);
fr, free tubular upper region ; *sh*, sheathing base of a leaf.   ×1.

CELLULAR ORGANISATION AT THE SHOOT APEX

The free apex of a vegetative bud of *Polygonum orientale* is rela-
tively low and broad, and is asymmetrical with reference to the longi-
tudinal axis (Fig. 2). A cross-section just a little below the extreme tip,
shows in the first plastochrone the central axis and the base of a leaf-
primordium in the form of a broad expansion on one side of the axis
(Fig. 3, *a–d*).

Above the last leaf-primordium, the free apex is composed en-
tirely of *eumeristem* (Fig. 2). The outer three or four layers form a
typical *tunica*, the cells of which divide only by anticlinal walls, and
within this is the *corpus*, a group of central initial cells, in which, parti-
cularly at the flanks, occasional periclinal divisions take place (Büder,
1928 ; Schmidt, 1924). The central initial cells are larger than the
other cells of the *eumeristem* ; the protoplasts appear finely vacuolated
and stain lightly. The *flank* meristem surrounds the central initials
, as a ring of tissue, broadest at the free surface of the apex and narrowest
near the pith-end of the central initial group. The cells are more regu-
lar than those of the central initials and the protoplasts are less
vacuolated. In the third internode near the base, the vacuolating
dividing cells tend to assume a *file* organisation and in the fourth and
fifth internodes from the apex, which are elongating vary rapidly,
these cells are fully organised in *files*, and their later divisions take place
exclusively by transverse walls. This region corresponds to the *Rippen*
meristem of Schüepp (1926).

The asymmetrical growth of the shoot apex is due to more intense activity in the sector which develops into the 'foundation' of the leaf-primordium, and comparatively less active growth in the rest of the apex.

ORIGIN OF LEAF-PRIMORDIUM AND ITS SHEATHING BASE

The localised activity of the *flank*-meristem causes an outward radial expansion of this particular sector of the shoot apex on a buttress of vacuolating meristem (Fig. 3, *c* and *d*). This is the first definite indication of the appearance of a new primordium. This radial expansion of the growing apex has been designated by Grégoire (1935) and Louis (1935) as "*Soubassement foliaire*", by Foster (1939) as "*foliar buttress*" and by Majumdar (1942) as "*foliar foundation*".

The *foliar foundation* is seen to surround the axis quickly by the lateral spread of similar divisions along the outer two or three layers of the *flank* meristem in the form of a collar. At the end of the second plastochrone the collar has completely surrounded the axis (Fig. 3, *d*). By this time the median prodesmogen strand (which develops later into the median bundle of the leaf-trace) is seen to differentiate in the middle of the *foliar foundation*, and it runs into the free portion of the primordium as it is erected. It may then be justifiably concluded that the emergence of the leaf-primordium is influenced by the basifugally differentiating vascular system from the adjoining part of the axis (*cf.* Esau, 1942, 1943 ; Majumdar, 1945).

DEVELOPMENT OF THE MAIN, LATERAL AND INTERMEDIATE VASCULAR STRANDS IN THE SHEATHING BASE

As stated above the median bundle of the leaf-trace appears as a prodesmogen strand for the first time in the second primordium. This is about $100\,\mu$ below the growing point. In the third primordium, 110μ below the growing point, near the level of insertion, the xylem is seen to differentiate in the median strand for the first time. Its further differentiation is both upwards in the free primordium and downwards in the axis. The median strand is then flanked by a lateral strand on either side (Fig. 4). Each of these lateral strands is then followed successively by two more lateral strands on each side, thus forming the second and third lateral pairs. An intermediate strand on each side then appears between the median and the first pair of laterals. Then follows the fourth pair of laterals. Between the second and the third laterals, on each side, now appear intermediates in succession. In this way fifth to ninth pair of laterals are differentiated one by one. Laterals of each opposite pair appear almost simultaneously. The sheathing bases comprising the wings and central region of the fourth and fifth primordia possess as many as 25 or 27 bundles. In older leaves further branching of the lateral and intermediate bundles is seen to take place and the total number of bundles in each sheathing base may reach 33 or more.
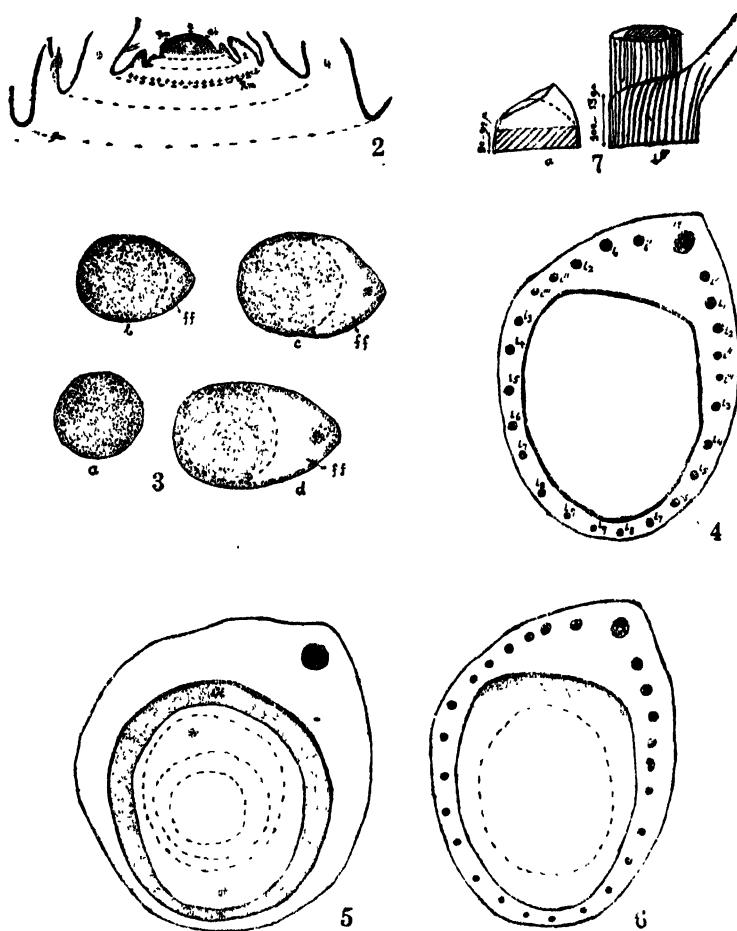
Figs. 2–7. *Polygonum orientale.*—Fig. 2. Longitudinal section of a vegetative bud showing shoot apex organisation. *t*, tunica ; *ci*, central initial ; *Fm*, flank meristem ; and *Rm*, rippen meristem. ×80. Fig. 3, *a–d*. Serial transverse sections of the vegetative apex showing free apex (*a*), the initiation of the foliar foundation (*b*), and its lateral extension along the free apex (*c* and *d*). *ff*, foliar foundation. ×150. Fig. 4. T.S. of the vegetative apex showing development of the lateral and intermediate bundles in the sheath. *L*, lateral ; *i*, intermediate ; and *M*, median. ×130. Figs. 5 and 6. Serial transverse sec tions of the vegetative apex showing separation of the sheath from the axis. Fig. 5 shows the " separation tissue " (*SL*), and Fig. 6 shows remnants of this tissue adhering to the free sheath. Fig. 5, ×300 ; Fig. 6, ×130. Fig. 7, *a* and *b*. Diagrammatic figure showing the growth of the sheath with the axis, then its separation from the axis, its free upgrowth, and its separation from the petiole,

FURTHER GROWTH OF THE SHEATHING BASE AND
ITS SEPARATION FROM THE AXIS

The sheathing base of the youngest primordium remains attached to the axis for 90–98 μ before it separates from the latter. Meanwhile the cells at the base of the third internode begin to divide actively. Soon the daughter cells organize into the *file* meristem and rapid elongation of the internode starts carrying the sheath with it.

While the internode is rapidly elongating a layer of vacuolating cells, opposite the median trace bundle of the sheath close to the axis, becomes meristematic and extends laterally around the axis to form ultimately the adaxial epidermis of the sheath. The walls of these cells become thickened and cutinised. The tissue lying between the axis proper and the newly formed adaxial epidermis of the sheath is composed of vacuolating parenchymatous cells, and is called here the 'separation layer' (*cf.* the *absciss layer* at the base of the leaf at the time of its fall), because this layer by its gradual disorganisation brings about the separation of the sheath from the axis (Fig. 5, *sl.*). It appears that the disorganization of the cells of the "separation layer" is brought about by two factors : (1) the cutting off of food supply from the sheath (which alone contains the conducting strands) due to the formation of cutinised adaxial epidermis, and (2) too much strain put on these cells on account of the very rapid extension of the leaf-primordium. The remnants of the disorganised " separation layer" remain in contact with the adaxial epidermis of the elongating sheath for some time (Fig. 6).

THE GROWTH OF THE UPPER FREE PORTION OF THE OCHREA
AND ITS SEPARATION FROM THE PETIOLE

The sheathing base pursues its free upward growth for 10 to 38 μ and then separates from the petiole to continue its upward growth as the free portion of the ochrea (Fig. 7, *a* and *b*). By the time the sheathing base begins its free growth upwards all the bundles of the sheath with the exception of the median one with its first pair of laterals (these enter the petiole direct), change their upward course and follow an oblique horizontal course towards the base of the petiole from the two wings of the sheath. In their horizontal or transverse course through the sheath the bundles unite and anastomose to form one or two irregular transverse strands (Fig. 8).

The organization of the transverse strands in the wings and petiolar region of the sheath may be regarded as the end of the *first phase* and the beginning of the *second phase* in the development of the ochrea in *Polygonum orientale*. If there had been no upward growth of the sheathing base beyond the petiole, the whole structure would have remained as the sheathing base only. But in this case, where the sheathing base is to develop as an ochrea, the upward growth is not arrested with the passing of the bundles of the sheath into the petiole, as happens in *Heracleum* and other plants with leaves having sheathing bases.

A little above the transverse strand in the petiolar region of the sheath, a few cells, opposite the median bundle and between the transverse strand and the adaxial epidermis, begin to divide rapidly by longitudinal walls. This results in the differentiation of a layer of meristematic cells, three or four cells below the epidermis (Fig. 9, *ml.*). This meristematic layer then gradually extends laterally towards the edges of the sheath which it reaches ultimately.

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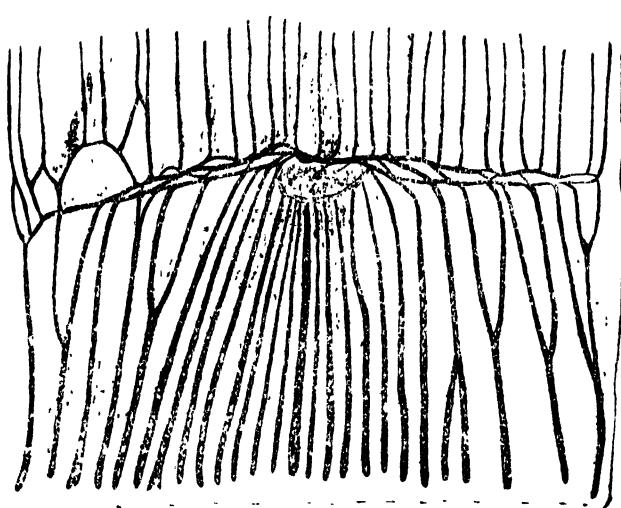
Fig. 8. *Polygonum orientale.*—An ochrea split open longitudinally, treated with chloral hydrate and stained in Safranin to show the course of vascular strands in the sheath and their branches in the upper free portion of the ochrea.   ×9.

While the meristematic layer is extending laterally, its cells towards the epidermis begin to expand radially pushing the cells in front and stimulating them to activity. As a result the sheath in front of this layer splits into two parts and for some time the split-ends move apart not being able to keep pace with the lateral extension of petiolar base (Fig. 9).

Meanwhile the meristematic cells within this redially expanding layer become gradually transformed into the adaxial epidermis of the petiolar base. This newly formed epidermis cuts off food supply to the radially extending cells immediately in its front, with the result that these cells disorganise. Thus a small cavity originates opposite to the median bundle ; this separates the adaxial epidermis of the petiole from the abaxial surface of the sheathing base now beginning to separate from the petiole (Figs. 10 and 11). The outermost cells of this region of the sheath then gradually change into the characteristic abaxial epidermis of the sheath.

The meristematic layer extends laterally till the petiole is entirely separated from the sheath (Figs. 12 and 14). The sheath now separated from the petiole grows upwards as the free upper portion of the

ochrea, and the split noticed in the early stages of separation and which is seen to grow from 10–30 μ in length is later closed. This split appears as the notch in the mouth on the posterior side of the fully developed ochrea (Fig. 9). It will thus be seen that the fork or notch does not indicate the composition of the ochrea as Asa Gray (1879, p. 106)
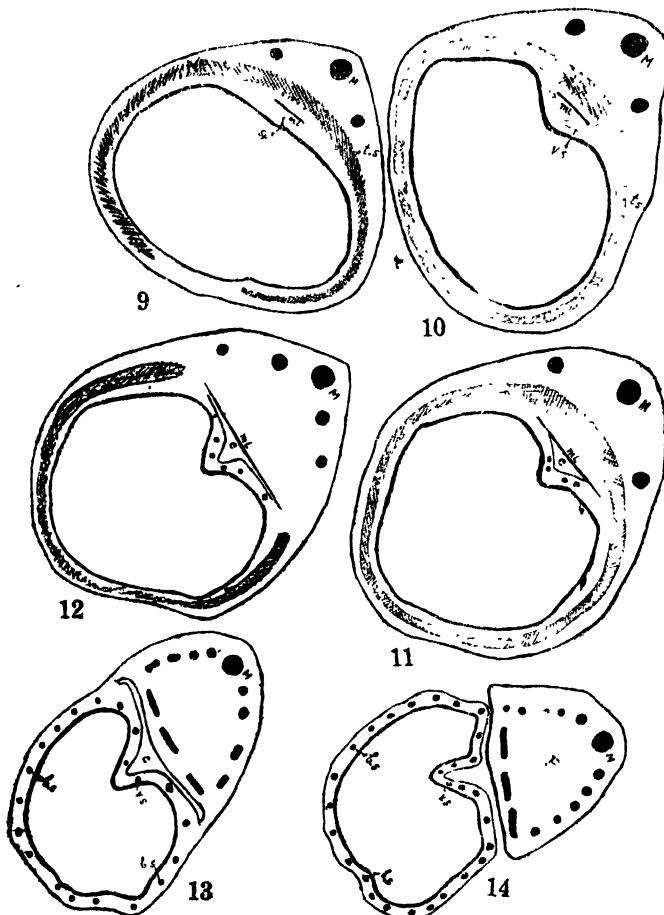
Figs. 9–14. *Polygonum orientale.*—Serial transverse sections of the vegetative bud showing separation of the ochrea from the petiole. Fig. 9. Origin of the posterior notch in the mouth of the ochrea ; shows appearance of a meristematic layer opposite the median bundle and the split caused by radial expansion of the tissue in front of the meristematic layer. ×120. Fig. 10. Shows the vascular supply from the petiolar base to the portion of the ochrea in front of the petiole. ×80. Fig. 11. Shows the formation of the cavity and the lateral extension of the meristematic layer. ×80. Figs. 12–14. Show gradual extension of the meristematic layer and the cavity resulting in the complete separation of the ochrea from the petiole. ×80. *ml*, meristematic layer ; *Sp*, split ; *M*, median ; *ts*, transverse strand ; *VS*, vascular strand from the petiolar base to the ochrea in front ; *c*, cavity ; *bs*, branch strand from the transverse strand to the ochrea ; and *P*, petiole.

would describe it, but is the result of pressure put on the sheath cells by the radially expanding cells of the petiole.

The stimulus which the cells of the separating portion of the sheath get from the radially expanding cells just in front of the meristematic layer, causes rapid growth and extension of this region and a fold soon appears opposite the petiolar base, evidently for accommodation of the upper part of the ochrea in the vegetative bud (Figs. 11–14).

VASCULAR SUPPLY OF THE OCHREA ABOVE THE BASE OF THE PETIOLE

While the bundles in the sheath are changing their course towards the base of the petiole and organising themselves into obliquely horizontal strands in the upper portion of the sheath, branches are given off from these strands (and in some cases also from the sheath bundles before these change their course) on the adaxial side of the sheath. Two or three bundles of the free upper portion of the ochrea which appear later in front of the petiole are secondarily derived from the transverse strand in the petiolar end of the sheath before it separates from the petiole (Figs. 8 and 10). The free upper portion of the ochrea gets about 22 to 25 bundles from the sheath, but in the mature condition the number is seen to increase to 29 or more due to the branching of these bundles in the free portion.

The free upper portion is comparatively thinner than the sheathing base. This appears to be due to the fact that the branches which are given out on the adaxial side are much slender than the sheath bundles from which they are given out.

DISCUSSION

The leaf of *Polygonum orientale* has a sheathing base, which originates independently of the nodal topography. Innervation of the base takes place in the second and third plastochrones. The upward growth of the sheath, till it separates from the axis, is due partly to the stimulus of growth and food material supplied through the large number of prodesmogen strands in different stages of differentiation. Further upward growth of the sheath is influenced, as suggested by Sinnott and Bailey (1914), by the stimulus of growth and food supply carried forward by the trace bundles till it is separated from the petiole.

In the case of leaves having sheathing bases alone, as in *Heracleum* and other plants, the lateral trace bundles in the sheath change their vertical course and run obliquely into the base of the petiole. The stimulus for upward growth of the sheathing base and food supply for influencing it being no longer available it remains as the sheathing base only. In the case of the ochrea of *Polygonum orientale* on the other hand, slender branches are given off from the lateral trace bundles during their united horizontal course towards and through the base of the petiole. These branches conduct the necessary food and stimulus for the continuance of the upward growth of the sheath, but in an attenuated form.

The view, most widely accepted and generally cited in text-books, about the morphology of the ochrea, which is a distinct feature of the leaf of *Polygonaceæ*, is that it represents two united stipules. This view is largely based on comparative morphology. The present investigation shows that the ochrea consists distinctly of two portions. The lower portion agrees in its development with the sheathing leaf-base such as is seen in *Heracleum* (*cf.* Majumdar, 1942). It is distinguished externally in *Polygonum orientale* as described above by its green colour. and prominent striations. The upper tubular portion is of the nature of an outgrowth from the leaf-base. As the stipules are generally regarded as outgrowths of the base of the leaf primordium (Sinnott and Bailey, 1914), the upper portion of the ochrea can also be interpreted to be of a stipular nature. The investigation, however, provides no evidence to show that it consists of two opposite stipules. It also contradicts the conclusion of Sinnott and Bailey (1914) that it is formed from a row of adjacent stipules each opposite one of the leaf trace bundles which have become fused together.

SUMMARY

The vegetative bud of *Polygonum orientale* has been studied from the organisational and developmental points of view. It shows that the ochrea has got two parts : (1) the sheathing base (which is found in many other groups of flowering plants) and (2) the free upper portion which develops as an outgrowth of the sheathing base and therefore, is to be interpreted as of stipular nature. The study does not support the conclusion of Sinnott and Bailey (1914) that the ochrea is a product of the fusion of a row of adjacent stipules.

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